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A Translation Of

PLANT PHYSIOLOGY

(FIZIOLOGIIA RASTENII)

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ASSIMILATION OF NUCLEIC ACIDS BY PLANTS AND EXTRACELLULAR PHOSPHATASE ACTIVITY OF ROOTS

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In our previous papers [1, 2], it was shown that assimilation in plants of organic compounds of orthophosphoric acid, which are products of the biological transformation of phosphorus in the soil under strictly sterile conditions, is closely related to the degree to which these compounds may be attacked by the extracellular phosphatase of roots. Of the organic phosphates tested, glycerophosphate and glucosephosphate were most easily attacked by the extracellular phosphatase of corn roots; they were also distinguished as the phosphorus source most available to the plants. Phytin is altogether inaccessible to the action of the extracellular phosphatase of corn roots, and in accord with this, phosphoric acid was not assimilated by corn under sterile conditions.

The exception to this rule is ribonucleic acid; it was noticeably decomposed by the extracellular phosphatase of corn roots, although much less so than glycerophosphate and glucosephosphate. However, as the sole source of phosphorus under sterile conditions, it gave only weak growth of corn and even had a toxic action. Meanwhile, the analysis of the experimental plants grown on ribonucleic acid showed an accumulation of considerable amounts of phosphorus, especially in roots, which could not be fully utilized for the construction of organic materials.

The reason for this was the sharply expressed formative action of ribonucleic acid, applied as the sole source of phosphorus on the root system of the plants, which retarded the normal growth of plants and interfered with the use of mineral phosphoric acid produced through the action of the extracellular phosphatase of roots.

In the case of loss of sterility in the root zone and the associated breakdown of ribonucleic acid by microorganisms, the plants developed quite normally, which permits one to assign the formative action on the root system to the ribonucleic acid itself, and not to products of its decomposition.

The following experiments, carried out in 1955, clarified certain details of this phenomenon. It was found that, depending on the environmental conditions (particularly upon the temperature conditions), the negative effect of ribonucleic acid as the sole source of phosphorus may reach different degrees of expression. Particularly under the cooler conditions of the first half of the growing period of 1955, the formative action of ribonucleic acid on the root system was expressed to a lesser degree than in 1954 and plants developed somewhat better, although considerably worse than plants which received phosphorus in mineral form (Fig. 1).

Thus, the method of sterile culture with simultaneous application of the entire dose of phosphorus into the root zone cannot serve as a reliable basis for clarification of the interaction between assimilation of ribonucleic acid by the extracellular phosphatase of roots and its availability to the plants as a phosphorus source. The question of the effect which externally applied nucleic acids exert on plants is of great interest at the present time. This is linked with the development of our study of the exclusive role of nucleic acid metabolism in plant life [3-5], on the one hand, and concerned with the significant participation of nucleic acid in the constitution of soil organophosphates [6-8], on the other.

Under natural conditions, the nucleic acids in soils undergo a constant turnover, either breaking down or being resynthesized in the life process of the biological agents in the soil. Because of this, plant use of this phosphorus source is doubly dynamic in nature. In connection with this, we decided in our further studies to

Fig. 1. Effect of ribonucleic acid as the sole source of phosphorus on corn plants under sterile conditions. On the left—mineral phosphorus; on the right—ribonucleic acid.

turn away from the method of giving plants the whole level of phosphorus at once and preferred to use the method of periodic fractionated nutrition, used often and successfully in the laboratory of D. N. Prianishnikov.

According to the principles of this method, we grew plants in the usual (non-sterile) water cultures on Knop's nutrient mixture without phosphorus (the potassium phosphate in Knop's mixture was replaced by potassium sulfate); more precisely, the solution contained an insignificant amount of phosphorus carried in the tap water from which the nutrient mixture was prepared. Phosphorus feeding of the plants was accomplished by placing the roots for 3 hours every other day into vessels containing various sources of phosphorus in low concentrations (2.5 mg of elemental phosphorus in 500 ml of water), at the same pH—5.2-5.3. After the three-hour period of phosphorus feeding, the plants were returned again to the vessels of Knop's nutrient mixture without phosphorus. The repeated feeding with phosphorus was always carried out with solutions freshly prepared immediately before the experiments. In this preparation, the ribonucleic acid was thoroughly ground with a small amount of water; after introduction into the vessel, it formed a very finely suspended non-precipitating suspension.

Under the above-described conditions of the experiment, the possibility of a formative action of ribonucleic acid on the root systems was eliminated altogether. Together with this, as control experiments have shown, during such short periods of phosphorus feeding (3 hours) the possibility of breakdown of ribonucleic acid by microorganisms was also eliminated.

Together with ribonucleic acid, the experiment included for comparison a mineral source of phosphorus (solution of KH_2PO_4, brought to pH 5.2-5.3), and glycerophosphate, a representative of organic phosphates easily attacked by extracellular phosphatase.

Corn plants, variety Beloiaroe psheno, were grown for four and a half months. The experimental results are shown in Fig. 2 and Table 1 (average of four replicates).

It is clear from Table 1 and Fig. 2 that under conditions in which the formative action of ribonucleic acid on the root system is eliminated, it is to some degree assimilated and used by plants as the sole source of phosphorus; however, to a much lesser degree than glycerophosphate. This is in accord with its being much

TABLE 1

Assimilation of Various Sources of Phosphorus by Corn Plants in the Experiment with Fractionated Nutrition

Phosphorus source	Dry weight per plant in g			Phosphorus content (P) in plants				
				In % of dry weight		In mg P per plant	Absorbed from phosphorus source	
	Aerial mass	Roots	Total	Aerial mass	Roots		In mg	In %
Mineral phosphorus	4.82	1.10	5.92	0.134	0.127	7.85	7.27	100
Glycerophosphate	4.30	1.14	5.44	0.107	0.140	6.19	5.61	78
Ribonucleic acid	2.51	0.51	3.02	0.071	0.127	2.42	1.84	25
Without phosphorus (control)	0.50	0.19	0.69	0.078	0.069	0.58	—	—

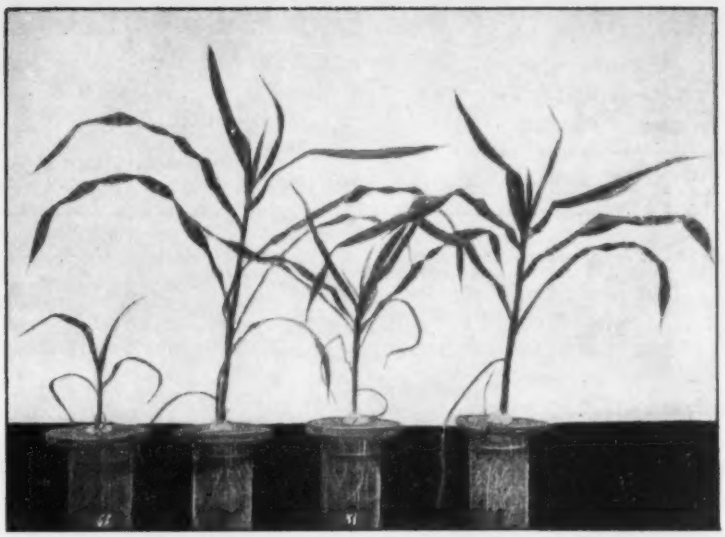

Fig. 2. Assimilation of various sources of phosphorus by corn in the experiment with fractionated nutrition. 1) Without phosphorus; 2) mineral phosphorus; 3) ribonucleic acid; 4) glycerophosphate.

Fig. 3. Availability of ribonucleic and desoxyribonucleic acids as the sole source of phosphorus to plants as compared with mineral phosphorus. The experiment with fractionated nutrition: 1) without phosphorus; 2) ribonucleic acid; 3) desoxyribonucleic acid; 4) mineral phosphorus.

less vigorously attacked by the extracellular phosphatase of corn roots [1]. With such an experimental setup, confirmation is obtained of the correspondence between the availability of ribonucleic acid to the plants as a phosphorus source, and the degree of its being attacked by the extracellular phosphatase of roots.

For the further clarification of this question, it seemed interesting to study the relative availability to plants of the two basic forms in which nucleic acids occur in nature—ribonucleic acid (RNA) and desoxyribonucleic acid (DNA)—comparing their availability with the degree to which these nucleic acids are attacked by the extracellular phosphatase of roots.

Such an experiment was set up with corn, using the methods of fractionated nutrition described above. Both ribonucleic acid and desoxyribonucleic acid were used in the form of highly purified preparations from

TABLE 2

Assimilation of RNA and DNA by Corn Plants as Sole Sources of Phosphorus, in the Experiment With Fractionated Nutrition (dry weight of 4 plants in g)

Source of phosphorus	Aerial mass	Roots	Total
Mineral phosphorus	15.23	5.51	20.74
Ribonucleic acid	7.44	3.08	10.52
Desoxyribonucleic acid	4.86	2.67	7.53
Without phosphorus (control)	1.93	1.17	3.10

Merck. The details of the experimental setup were the same as described above.

The external view of the experimental plants, shown in Fig. 3, as well as data calculated on the basis of dry weight after termination of the experiment (Table 2), clearly show the low availability of both RNA and DNA to plants as the sole source of phosphorus. This experiment, as was indicated above, was carried out at pH 5.2-5.3.

TABLE 3

Phytase and Glycerophosphatase Activity of the Roots of Corn and Pea (inorganic phosphorus (P) found in mg/ cm^3 of roots after 3 hours)

Substrate	7-day-old plants		25-day-old plants	
	Corn	Pea	Corn	Pea
Phytin	0.005	0.037	0.040	0.088
Glycero-phosphate	0.171	0.190	0.300	0.242
Water (control)	0.008	0.011	0.060	0.061

TABLE 3a

Inorganic Phosphorus (P) in mg/ cm^3 of Root Found After 3 Hours

Substrate	
Glycerophosphate	0.195
Ribonucleic acid	0.019
Desoxyribonucleic acid	0.018
Water (control)	0

In a parallel series of vessels, the same forms of phosphorus were presented to the plants under the same conditions at pH 8.0-8.2. However, at such a low phosphorus level as we used, the antagonism of OH ions in relation to phosphate ions was so sharply expressed that not only the phosphorus of nucleic acid, but even mineral phosphorus was not used at all by the plants. The weight of plants receiving phosphorus was not different from the weight of plants grown without phosphorus.

The results obtained are in agreement with the following data which indicate a very weak activity of the extracellular phosphatase of corn roots toward both RNA and DNA, as compared with its activity toward glycerophosphate, which was taken as a standard.

For measurement of the activity of extracellular phosphatase of roots on the organic phosphates mentioned, the roots of young corn plants grown on nutrient mixture without phosphorus were used. Such plants are characterized by the most active extracellular phosphatase of their roots. The method of calculating the phosphatase activity of the cut strands of roots was described in one of the previous papers [1].

From the content of mineral phosphorus in solution after a 3-hour period of interaction of the roots with the corresponding organic phosphates we subtracted the insignificant amount of mineral phosphorus which was found in such samples of the initial preparation after they had stood for a period of three hours under the same conditions, but without roots; the resulting figures are given in Table 3a.

The interconnection between the availability of a particular organic phosphate as a phosphorus source for plants and the activity of extracellular root phosphatase of a given plant on the phosphate may be illustrated in still another case: that of phytin.

In one of our previous papers [2], it was shown that phytin is practically unassimilated by corn under sterile conditions. The same was also observed in an old experiment of Shulov [9]. Meanwhile, in his own experiments the pea plant assimilated phytin to a considerable degree, although much less than mineral phosphorus. In connection with this, it was interesting to compare the extracellular phytase activity of roots in pea and corn with their capacity for assimilation of this form of organic phosphorus.

Corresponding data, obtained with the roots of young plants of pea and corn, are given in Table 3. Glycerophosphate was also included in the experiment for comparison, as a form of organic phosphorus easily available to plants.

As can be seen from Table 3, the pea roots are able to split phosphorus from phytin, although in small

TABLE 4

Effect of the Condition of Mineral Nutrition on Phosphatase Activity of Corn Roots (inorganic phosphorus (P) found in mg/ cm^3 of roots after 3 hours)

Nutrition conditions during previous growth of experimental plants	Roots placed in solution of glycerophosphate	Roots placed in distilled water (control)	P split from glycerophosphate
Complete Hellriegel's nutrient mixture	0.234	0.037	0.194
Without N	0.343	0.368	0
Without K	0.263	0.252	0.011
Without P	0.312	0.002	0.310
Tap water	0.055	0.001	0.054

amounts, while the corn roots did not show this capacity to any degree at all (the phosphorus content in the control is even somewhat higher than with phytin). At the same time, the decomposition of glycerophosphate is accomplished by corn roots at a rate not less than that of pea roots, in accordance with the high availability of this organic phosphate to all plants.

Thus, the combination of all the data mentioned again emphasizes, through the examples of assimilation of nucleic acid and phytin by plants, the significance of extracellular, mainly surface [1],phosphatase activity of roots as a powerful factor by which the plant actively uses its environmental medium for the purpose of satisfying its needs for mineral substances. Activity of the surface phosphatase of roots toward a particular organic orthophosphate turned out to be a reliable symptom, characterizing the direct availability of this compound as a source of phosphorus for plants.

One must say that at the present time the concept of surface enzyme activity of living cells and tissues, both plant and animal, receives greater and greater recognition in various areas of enzymology [10]. Applying this phenomenon to the question of plant nutrition is all the more interesting because it presents a new aspect to the general picture of an active reaction of the plant in the zone of contact between its roots and soil particles.

Another very interesting fact, discovered during our further investigations, is that the phosphatase activity of roots finds its greatest expression when the plant, supplied with all other elements of nutrition, is strongly deficient in phosphorus. This emphasizes the adaptive significance of the observed link in the metabolic activity of roots, which allows the plant to convert organic phosphorus compounds in the soil to its own use, when available mineral forms of these elements are lacking in the zone inhabited by roots; such mobilization depends upon the degree of the deficiency. This is illustrated by the experimental results with corn. The results of the experiment are given in Table 4.

Corn plants were grown in water culture on Hellriegel's nutrient mixture, omitting nitrogen, phosphorus, or potassium. The nutrient mixtures were prepared with tap water, so that the plants had only the small amounts of these elements which are contained in tap water. Along with omission of individual elements from the nutrient mixture, there was included in the experiment a treatment with general nutrient starvation of plants (tap water alone, without addition of nutrient salts), as well as a treatment with full Hellriegel's nutrient mixture. When the starvation symptoms were readily seen in the plants, the roots were taken for the measurement of their phosphatase activity toward glycerophosphate.

It can be seen from Table 4 that roots of the plants given a normal amount of phosphorus, with a strong deficiency of nitrogen and potassium, released phosphorus after being placed in distilled water, and after being placed in solution of glycerophosphate show no (without N), or almost no (without K), phosphatase activity.

The phosphatase activity of roots in plants starved for all nutritional elements (grown on tap water alone) is also insignificant. The greatest phosphatase activity was shown by the roots of those plants which were starved only for phosphorus. Significant, although to lesser degree, phosphatase activity was shown by plant roots which received a complete nutrient mixture.

Thus, as was indicated in our preliminary experiments [1], the phosphatase activity of roots depends to a

Fig. 4. Effect of a stimulating dose of ribonucleic acid on growth of corn in sterile cultures, against the background of mineral phosphorus. At left—complete Knop's nutrient mixture; on the right—the same, but with replacement of 10% of mineral phosphorus by ribonucleic acid.

significant degree on the conditions of plant nutrition, foremost among which is the degree of mineral phosphorus supply. We are inclined to see in this the explanation of the fact that insofar as glycerophosphate, which is easily attacked by roots, is concerned, its decomposition is not to completion [1, 2]. As the plants' need for phosphorus is satisfied, and mineral phosphoric acid is accumulated in the zone inhabited by roots, the phosphatase activity of roots is decreased, especially during a relative deficiency of nitrogen and potassium.

To return to the question of the assimilation of nucleic acids by plants, it should be emphasized that in the experiment described above, we were concerned with the availability of these acids as the sole source of phosphorus for the plants. With such a setup, there is hardly any basis, as we saw, for exaggerating the significance of nucleic acid as a direct source of phosphorus for plants [11]. In accordance with the low degree of activity of the extracellular phosphatase of roots toward nucleic acids, the latter are, excluding the help of microorganisms, significantly inferior in their effectiveness for plants. This is true for mineral phosphate and the easily attacked organic phosphates, such as glycerophosphate.

However, it is possible to look at the importance of nucleic acids to plants in another way. We have in mind their possible significance as physiologically active substances, showing their affect on plants when present in insignificant amounts, aside from their role as a source of phosphorus, i.e., when they are introduced against a background of a completely sufficient phosphorus supply in the mineral form which is easily available to plants. For us, the basis for such a question was the fact, established in our laboratory, that many products of the biological turnover of nitrogen and phosphorus in the soil (such as vitamins and a number of amino acids, as well as organic phosphates — nucleic acids among them [2]) may in some way be absorbed by roots in a changed form, and show their effect on plants in accordance with their physiological activity. Thus for example, the B-group vitamins have shown a clearly expressed favorable effect on the synthetic activity of roots in connection with the primary transformation of mineral nitrogen [12]; some amino acids, especially asparagine and glutamine, favored a better growth of roots in their intense branching [13]. Phytin, practically unvailable as a source of phosphorus for corn, nevertheless, in small amounts, gets into the root system in a changed form, and favors a better development of roots of this plant in sterile cultures [2].

Upon considering the high physiological activity of nucleic acid which is particularly shown, as was

TABLE 5

Effect of a Small Dose of Ribonucleic Acid, Introduced Against the Background of Knop's Complete Nutrient Mixture, on Growth of Corn in Sterile Cultures

(average of three replicates)

Experimental treatments	Dry weight per plant in g			Root volume in cm^3
	Aerial mass	Roots	Total	
Knop's complete nutrient mixture	17.61	2.37	19.98	45.3
The same, but with replacement of 10% of mineral phosphorus by ribonucleic acid	20.69	3.00	23.69	52.6

mentioned before, in a sharply expressed formative effect on the root system when introduced as the sole source of phosphorus in sterile cultures, it seemed interesting to clarify its effect on the same plant when applied in small doses against a background of normal plant nutrition with mineral phosphorus. Such experiments were carried out with corn both by the method of sterile cultures and by the method of periodic fractionated nutrition.

The experiments in sterile cultures were carried out in such a way that, along with the vessels where phosphorus was applied in mineral form, i.e., as KH_2PO_4, as it should be in Knop's mixture, there were also vessels in which 10% of the total amount of phosphorus was replaced by ribonucleic acid with subsequent adjustment to the same pH value (about 5.5).

The Knop's mixture in all cases was used at a somewhat reduced concentration (75% of the full level). In accordance with this, to replace 10% of the total phosphorus level with ribonucleic acid, the latter had to be introduced in the amount of 225 mg per vessel containing 5 liters of nutrient solution.

Since, when the KH_2PO_4 level was decreased by 10%, the potassium level was also decreased, the latter was added in the form of KCl.

TABLE 6

Effect of a Small Dose of Ribonucleic Acid, Introduced on a Background of Knop's Complete Nutrient Mixture, on the Life Activity of Corn Root in Sterile Cultures

Experimental treatment	Amount of sap obtained, in g		Content of phosphorus in the sap, in mg				Content of nitrogen in sap, mg		Content of amine and amide nitrogen, in mg	
			Per ml of sap		In total flow of sap					
	Calculated per cm^3 of root	Calculated for the whole root system	Total	Inorganic	Total	Inorganic	Per ml of sap	In total flow of sap	Per ml of sap	In total flow of sap
Knop's complete nutrient mixture	0.196	8.78	0.0073	0.0076	0.063	0.067	0.0048	0.039	0.0285	0.259
The same, but with replacement of 10% of mineral phosphorus by ribonucleic acid	0.392	20.49	0.027	0.0295	0.604	0.657	0.0170	0.3545	0.0786	1.611

The details of the sterile culture method we applied and the method of introduction of ribonucleic acid may be found in previous papers [1, 2].

Throughout the whole period of plant growth it was seen that addition of the small dose of ribonucleic acid against the background of normal mineral phosphorus nutrition not only gave positive results, but noticeably stimulated plant growth and development (the earlier appearance of tassels, earlier anthesis, the earlier beginning of ear formation). No negative action on the root system was found (Fig. 4).

The data on the dry weight of plants and on their root volume are given in Table 5. These data refer to the vessels in which the medium inhabited by roots was sterile until the end of the experiment. The plants were harvested in the phase of beginning of ear formation.

One may obtain some idea of the effect of a small addition of ribonucleic acid on the life activity of a root system from the data showing the rate of nitrogen and phosphorus movement with the sap flow in the roots of the experimental plant, as well as from the rate of secretion of the sap itself (Table 6).

The sap was collected under sterile conditions by means of collectors with siphons, used for this purpose in our laboratory [14].

From the data of Table 6, a sharp distinction can be seen in the level of root life activity of plants which

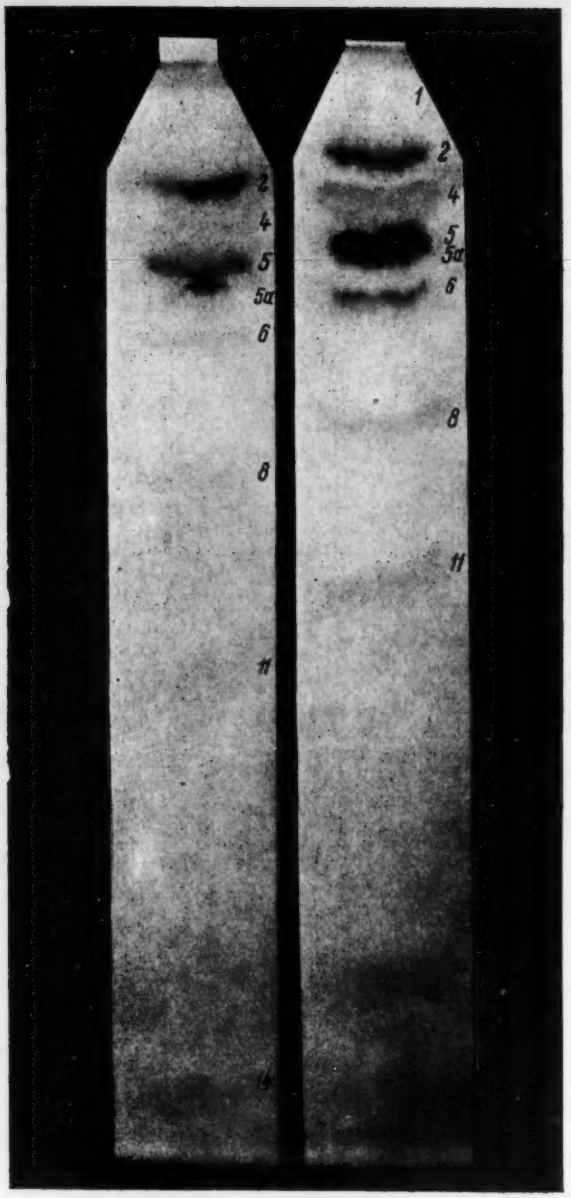

Fig. 5. Chromatograms of amino acid composition of the sap in corn plants grown in sterile cultures. On the left—on Knop's complete nutrient mixture; on the right—the same, but with replacement of 10% of mineral phosphorus by ribonucleic acid. Amino acids: 1) cystine; 2) lysine; 4) asparagine; 5) arginine; 5a) aspartic acid; 6) serine; 8) threonine; 11) alanine; 14) valine.

did or did not receive a stimulating dose of ribonucleic acid against the background of the normal supply of mineral phosphorus. In the former case, one notes a sharply increased rate of secretion of the sap itself and a sharply increased concentration of compounds of nitrogen and phosphorus in this sap.

We shall note, in connection with this, that the recent work of Tanada [15] showed a sharp decrease in

TABLE 7

Effect of Replacement of a Small Part of the Mineral Phosphorus by Ribonucleic Acid on the Growth of Corn and its Use of Nitrogen and Phosphorus From the External Medium (experiments with fractionated nutrition; plants were grown to the age of 45 days; average of 4 determinations)

Phosphorus source	Weight per plant in g			Total N content in plant				Total P content in plant			
				In % of dry weight		Total per plant		In % of dry weight		Total per plant	
	Aerial mass	Roots	Total	Aerial mass	Roots	In mg	In % of control	Aerial mass	Roots	In mg	In % of control
All phosphorus in mineral form (control)	4.82	1.10	5.92	2.05	2.00	120.8	100	0.134	0.127	7.85	100
90% of phosphorus in mineral form plus 10% in the form of ribonucleic acid	5.68	1.35	7.03	2.22	2.10	154.5	128	0.126	0.143	9.09	116

the absorption of phosphate ions by the cut roots of bush beans under the effect of ribonuclease treatment. This also indicates the close connection between the absorbing activity of roots and their nuclear metabolism.

Addition of ribonucleic acid was not without effect on the amino acid composition of the sap; under the influence of ribonucleic acid, the content of a number of amino acids, particularly asparagine, aspartic acid, and serine, is considerably increased (Fig. 5).

Chromatograms were made using butanol: water: formic acid, in the ratio 18: 9: 2, as solvents, and using ninhydrin as a spray.

Identification of amino acid was carried out with the aid of control standards, and in addition, by means of staining parallel chromatograms with isatin which yields characteristic colorations with the different amino acids [16].

Attention is drawn to the fact that, in Table 6, all the phosphorus in the sap of both control and experimental plants is represented by the inorganic form. The same results were obtained in analyzing the sap of plants which receive higher doses of ribonucleic acid as the sole source of phosphorus: no organic phosphorus was found in the sap of these plants.This phenomenon makes it difficult to explain the effect of a small addition of ribonucleic acid on the growth, and especially on the development, of plants by an increased supply of nucleic acids from the roots to aerial organs, which, according to the proposal of Sabinin [17], supported recently in the work of Potapov and Maroti [18], is an important link in the interrelationship of roots and aerial plant organs.

The data of Table 6 indicate rather that the action of ribonucleic acid on the growth and development of plants takes place through its effect on the life activity of the root system itself; thus, the effect concerns not only phosphorus but also nitrogen metabolism. However, the possibility is not excluded that a certain role is played here by the roots in supplying the aerial organs, not with ribonucleic acid in unchanged form, but with a breakdown product not containing phosphorus, particularly with those purine and pyrimidine bases which are parts of nucleic acid and which, on entering the leaves, show an effect on the whole process of nuclear metabolism there. These problems need further elaboration.

Let us now turn to the results of the experiment using the method of fractionated nutrition. As was already pointed out, in these experiments the phosphorus was given to the plants in low concentration (2.5 mg P per 500 ml of solution). In accordance with this, when 10% of the mineral phosphorus was replaced by ribonucleic acid, the latter was introduced only in the amount of about 3 mg per 500 ml of solution, i.e., in a concentration almost one-eighth that of the concentration in the experiment described above. Nevertheless, the positive effect of this small addition of ribonucleic acid on plant growth, plant development (earlier

TABLE 8

The Effect of Replacement of a Small Part of the Mineral Phosphorus by Ribonucleic Acid, and Also of the Total Replacement of Mineral Phosphorus by Ribonucleic Acid, on Phosphorus Metabolism in Roots and Aerial Organs of Corn

Phosphorus source	Aerial mass				Roots			
	Total P content in % of dry weight	Content of separate forms of P in % of total			Total P content in % of dry weight	Content of separate forms of P in % of total		
		P of nucleo-proteins and phos-phatides	Acid-soluble inorganic	Acid-soluble organic		P of nucleo-proteins and phos-phatides	Acid-soluble inorganic	Acid-soluble organic
All phosphorus in mineral form	0.134	34.3	58.2	7.5	0.127	46.4	52.0	1.6
10% of phos-phorus in the form of ribonucleic acid	0.126	27.0	67.5	5.5	0.143	53.8	33.6	12.6
Mineral phos-phorus	0.134	34.3	58.2	7.5	0.127	46.4	52.0	1.6
Ribonucleic acid 100%	0.071	38.0	60.6	1.4	0.127	51.2	37.0	11.8

tasseling), and also the plant's use of nitrogen and phosphorus from the external medium was quite noticeably expressed in this experiment.

This is illustrated by the data in Table 7.

From the data of Table 7 one sees the positive effect of a stimulating dose of ribonucleic acid, not only on the absolute accumulation of nitrogen by plants, but also on its relative content, both in aerial organs and in roots. Along with this, the analysis of plants for protein nitrogen content showed that protein synthesis was increased under the influence of ribonucleic acid, especially in the roots (the protein nitrogen content was 73.8% of the total, instead of the 69.5% in the roots of control plants). All this, in addition to the data given above in the experiment with sterile cultures, demonstrates the positive effect of the added microdose of ribonucleic acid on nitrogenous (protein) metabolism in plants, especially in roots. Such a conclusion is in accord with the generally accepted concept of the exceptional role of nucleic acid, in particular RNA, in protein synthesis [3-5, 19, 20].

Addition of a microdose of RNA also had its effect on phosphorus metabolism in the plant, which can be judged by the results of analysis of experimental plants for the content of certain forms of phosphorus according to the scheme of Sokolov [21].

As can be seen from the data of Table 8, a sharp increase in the acid-soluble organic phosphorus fraction is noticed in plant roots, under the influence of RNA, at the expense of a decrease in the inorganic phosphorus fraction. Along with this, a certain increase in the fraction of nucleoprotein and phosphatide phosphorus is also noted in roots. In the aerial mass, the changes are less sharp and are reversed in direction.

Similar data on the content of these same forms of phosphorus were obtained also in an analysis of the roots and aerial mass of plants grown on ribonucleic acid as the sole source of phosphorus in the experiment of fractionated nutrition, the results of which were given in Table 1.

The data of these analyses are also given in Table 8.

Here too, the ribonucleic acid caused a sharp increase in the fraction of acid-soluble organic phosphorus in roots, and a decrease in the inorganic phosphorus fraction. Here the picture is reversed in the aerial mass. Under these conditions the increase in the fraction of nucleoprotein and phosphatide phosphorus under the influence of RNA is again repeated in the roots. It was not possible to analyze this phenomenon any further with the present analytical material. However, one cannot doubt the effect of RNA on the increase in the synthesis in the roots of the phosphoric esters which play such an important role in the whole biodynamics of living cells and tissues.

SUMMARY

1. The low efficiency of ribonucleic as well of desoxyribonucleic acid as a direct source of phosphorus for maize plants was demonstrated by the method of fractional nutrition which permits one to completely overcome the formative influence (previously mentioned) in sterile cultures of ribonucleic acid on the root system of plants. In agreement with the fact that these nucleic acids are affected to a weaker extent by extracellular phosphatase of maize roots, their efficiency was found not only to be below that of mineral phosphorus but of glycerophosphate as well, which is much more easily affected by extracellular phosphatase of the roots.

2. Extracellular phosphatase activity of roots depends to a large extent on the conditions of mineral nutrition of the plants. No appreciable phosphatase activity was detected in roots of maize plants well supplied with phosphorus but deficient in nitrogen or potassium. It was likewise insignificant in plants which were insufficiently supplied with all the various nutrients. Roots of plants sufficiently supplied with all required nutrients exhibited a considerable extracellular phosphatase activity. However, the highest activity was observed in plants deficient only in phosphorus.

This circumstance emphasizes the adaptative role of the process under consideration in the metabolic activity of roots; this process permits the plant to mobilize and subsequently utilize organic phosphorus compounds in the soil when the latter does not contain a sufficient amount of mineral forms containing accessible phosphorus.

3. If nucleic acids, which are inefficient as direct phosphorus sources, are supplied in insignificant amounts against a background of an abundant supply of phosphorus, a pronounced stimulating effect on the root system and, ultimately, on the growth and development processes of the plant is observed. Microdoses of ribonucleic acid enhanced the synthesis of protein and phosphoric esters in the roots, increased the nitrogen and phosphorus content in the sap. Excretion of the latter and its amino acid content, especially the amount of aspartic acid and serine, increased.

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THE INFLUENCE OF SOIL TEMPERATURE ON GROWTH, DEVELOPMENT AND YIELD OF OATS UNDER VARIOUS CONDITIONS OF MINERAL NUTRITION

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For a long time the significance of soil temperature was either not taken into account at all in experiments with plants, or its effect on the life processes was equated with that of the air temperature. In investigations carried out to clarify the role played by soil temperature in the life of plants [1-6 and others], the different temperature adaptability of aerial organs and roots was not taken into account.

The question of the soil temperature effect on plants, from the point of view of temperature gradients, i.e., the significance to the plant of a difference in temperatures between air and soil, was first studied by Radchenko [7, 8]. The roots of higher plants are historically adapted to lower temperatures than are the aerial organs. Because of this, plants grow and develop well under conditions of a negative temperature gradient, i.e., when the temperature of the soil or nutrient solution is lower than the temperature of the air. S. I. Radchenko notes the great significance of the original temperatures forming the gradient.

The problem in the present work was to clarify the way in which various soil temperatures under various conditions of mineral nutrition affect respiration, carbohydrate and chlorophyll accumulation, and also development and yield of oats.

METHODS

The experiments were carried out at the Biological Station of the A. P. Gertsen State Pedagogical Institute in Vyritsa of the Leningrad Region.

Oats, variety Zolotoi dozhd', were grown in "vegetative houses" constructed by S. I. Radchenko, designated "heated house" and "unheated house."

In the heated house, the pots with plants were placed on wooden boards under which electric lamps were placed (30 lamps, 100 watts each) in three rows, checkerboard fashion. When it rained, the houses were covered with greenhouse frames; the rest of the time (both day and night) they were open. A similar arrangement of houses was described by Radchenko [9]. There are also given data concerning soil temperatures in the heated house, depending on various external temperature conditions, and also a graph characterizing the dynamics of the air and soil temperatures etc., taken from the investigations described in the present paper. The differences in the soil temperatures in the heated and unheated houses was, on the average, 4.7° throughout the vegetative period. The average temperature of the soil in the pots and of the air are given in Fig. 1.

The plants of both series were divided into seven treatments, depending on the fertilizer doses applied. Normal Hellriegel's solution was used as the basic nutrient mixture, and its elements (N, P, K) were varied in corresponding treatments of the experiments:

I) Control, without fertilizer; II) N_1 (one dose of nitrogen); III) $N_1P_1K_1$ (one dose of nitrogen, phosphorus, potassium); IV) $N_1P_3K_3$ (one dose of nitrogen, three doses of phosphorus, three doses of potassium); V) N_3 (three doses of nitrogen): VI) $N_3P_1K_1$ (three doses of nitrogen, one dose of phosphorus, one dose of potassium); VII) $N_3P_3K_3$ (three doses of nitrogen, phosphorus, and potassium).

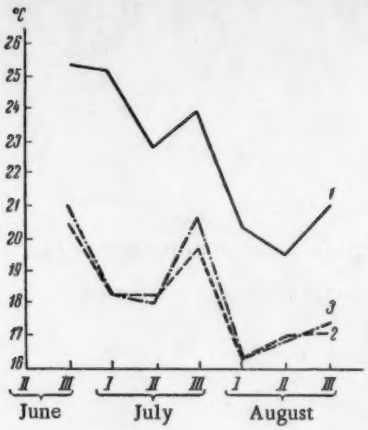

Fig. 1. Average daily temperature of the air and of soil in pots by 10-day intervals in the vegetative period, 1953. 1) Heated; 2) unheated; 3) air temperature.

The chlorophyll content, soluble carbohydrate content, and respiration rate were determined in the leaves of plants of treatments I, II and V of both series. The determination of chlorophyll in leaves was carried out by Liubimenko's spectrocolorimetric method [10]. Monosaccharides and sucrose were determined according to carbon dioxide evolved (Kursanov's method).

EXPERIMENTAL DATA

The Rates of Oat Development

Plant development speeded up at an increased temperature; in all treatments in the heated series the plants matured 10 to 12 days earlier. However, the different soil temperature has a different effect in the course of ontogenesis both on the onset of phenological phases and on the effectiveness of various fertilizer doses at various phases. The tillering phase takes place earlier in all unheated treatments, and no effect of various doses of fertilizer was found at this stage. The shooting phase, as well as that of tillering, takes place somewhat earlier in the unheated treatment in all phases with mineral nutrition, but after this phase, a break occurs in the development of the plants. A prolongation of the period from the shooting phase to panicle formation is clearly seen in unheated plants, and a shortening of this period is seen in the heated series. In this period also, the effect of fertilizers at various soil temperatures is also noticeable. For example, in the heated series with three doses of nitrogen (treatment V), panicle formation occurs 12 days after the shooting phase, and in the same treatment in unheated series, it occurs 19 days after shooting. The same may be observed in the other treatments of the heated and unheated series (Table 1).

TABLE 1

Duration of the Period From the Shooting Phase to Panicle Formation (in days) in Oats, Variety "Zolotoi dozhd'"

Experimental treatments	Heated	Unheated
Control	15	20
N_1	15	20
$N_1P_1K_1$	12	17
$N_1P_3K_3$	15	19
N_3	12	19
$N_3P_1K_1$	13	18
$N_3P_3K_3$	15	21

As Skazkin [11] noted, there is a reorganization in the plant organism during the period of formation of sexual organs, connected with the transformation from vegetative development to reproductive development. This reorganization evidently makes the plant more sensitive to the effect of external factors. Because of this, the period from the shooting phase to panicle formation (more precisely, the period from tetrad formation to fertilization, inclusively) is very sensitive to the effect of a positive temperature gradient, as well as to a deficiency of water in the soil (Skazkin [12]), illumination intensity (Novikov [13]), etc. Beginning with the phase of panicle formation, development occurs more rapidly in all heated treatments, which leads to the earlier maturation of these plants. According to our data [14], the acceleration of development is particularly favored by warming of the soil during the light stage. In both the heated and unheated series, the development of oats was retarded when a high level of nitrogen was applied to the soil. However, the vegetative period of plants of the same treatment in both series was shorter in the heated series than in the unheated series. Consequently, although high levels of nitrogen with increased soil temperature still retard the development of plants, they do not retard development as noticeably as at a normal temperature.

Nitrogen acts specifically in the soils of various temperatures [15, 16], which is reflected in the dependence of the rate of plant development on the levels of nitrogen applied to the soil. It is possible that the data obtained by Chailakhian [17], indicating a dissimilar action of excessive levels of fertilizer on various plants, are connected with the historical adaptation of plants to different temperatures. Thus, an excess of nitrogen

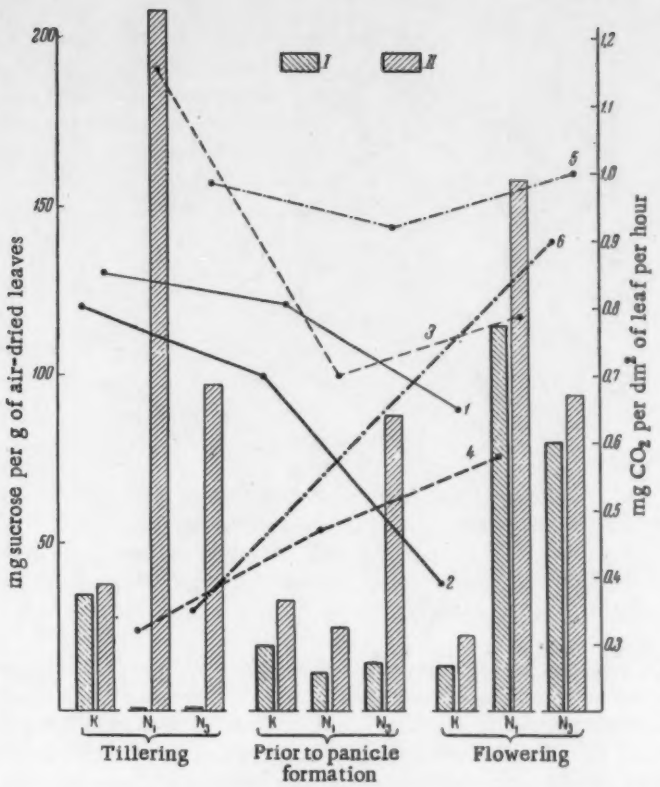

Fig. 2. Change of respiration rate of oat leaves, variety "Zolotoi dozhd'," and sucrose content in the leaves at various soil temperatures and nitrogen levels. I) mg sucrose in plant leaves in the heated series; II) mg sucrose, unheated series; 1) unheated control; 2) heated control; 3) N_1, unheated; 4) N_1, heated; 5) N_3, unheated; 6) N_3, heated.

fertilizer retards the development of oats – a plant of temperate latitudes, at the same time that it accelerates the development of millet, perilla, and soya – heat-loving plants.

Carbohydrate Metabolism and Respiration

The data we obtained show (Fig. 2) that considerably more soluble sugars, especially sucrose, are accumulated in oat leaves in the unheated than in the heated series. An accumulation of sucrose indicates an abundance of synthetic processes [18] and a heightened living tonus of the plant.

In connection with the large accumulation of sucrose in leaves, their respiration rate is higher in the unheated series (Fig. 2). These data agree with the experimental results of L'vov [19] and others, who have shown that the curve of respiration most closely follows the curve of sucrose, repeating its inflections.

The maximum amount of sugar in both series of experiments occurs in the period of panicle formation, and particularly in the phase of flowering, which must be associated with the increase of general physiological activity during this period. The control treatments (without fertilization) of both series were exceptions in which the soluble sugar content in leaves was lowered with age, evidently due to nitrogen starvation.

Chlorophyll Content in Oat Leaves

The study of chlorophyll content in ontogenesis of oats has shown that more chlorophyll is formed in all treatments of the unheated series (except the control) than in plants of the heated series (Fig. 3). According to

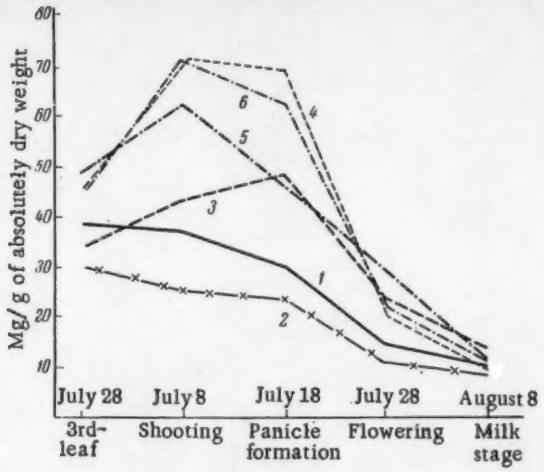

Fig. 3. Dynamics of chlorophyll in the fourth leaf of oats, variety "Zolotoi dozhd'," as affected by the soil temperature and nitrogen levels. 1) Heated control; 2) unheated control; 3) N_1, heated; 4) N_1, unheated; 5) N_3, heated; 6) N_3, unheated.

the opinion of some authors [20, 21], the soluble sugars take an active part in the formation of the chlorophyll molecule. Evidently, the increased chlorophyll content in leaves of unheated plants is explained by this. The treatments without fertilizer (control) were exceptions; more chlorophyll was formed in the heated series than in the unheated series. This evidently takes place because of the better mobilization of nitrogen in the soil at the increased temperature. In the treatments which were well supplied by nitrogen, the maximum chlorophyll was observed during the period from shooting to panicle formation, after which the amount of chlorophyll decreased. The maximum chlorophyll formation in the control treatment took place at the very earliest phase of development. Consequently, the location of the chlorophyll maximum was not constant, even in the plants of one species, but changed in accordance with the nutritional conditions: the more poorly the soil was supplied with the elements of mineral nutrition, the earlier the fall of chlorophyll content took place in the leaves of the plant, since the plant begins earlier to experience a deficit of nutritional substances, particularly of nitrogen. With an increase of nitrogen level, the amount of chlorophyll increases, both in the heated and unheated series (Fig. 3).

Yield of Experimental Plants

It is known [18, 19] that a relative predominance of synthetic processes is characteristic of late-maturing varieties. This leads to a significant increase of sugar content, particularly of sucrose, in the leaves and is accompanied by an increase in oxidative reaction activity, as well as of respiration.

In the leaves of plants of the unheated series, considerably more soluble sugars, particularly sucrose, are accumulated, the respiration rate is greater, and the amount of chlorophyll is larger than in the heated series. All this allows one to conclude that the plants of the unheated series, in which the development was slowed down in comparison with the plants of the heated series, develop like a late-maturing type, while the plants of the heated series develop like an early-maturing type.

The effect of various soil temperatures on the yield of experimental plants is opposite to their effect on development rates. Thus, if plant development was more rapid in all treatments of the heated series, then the yield was higher in all treatments of the unheated series, with some exceptions. The effect of soil temperature on the accumulation of aerial mass is already visible prior to panicle formation. Thus, the weight of the aerial mass was higher in all treatments of the unheated series than in corresponding treatments of the heated series. During the period of shooting and panicle formation, the plants are sensitive to the effect of higher soil temperature, not only in relation to the acceleration of development, but also with regard to the accumulation of aerial

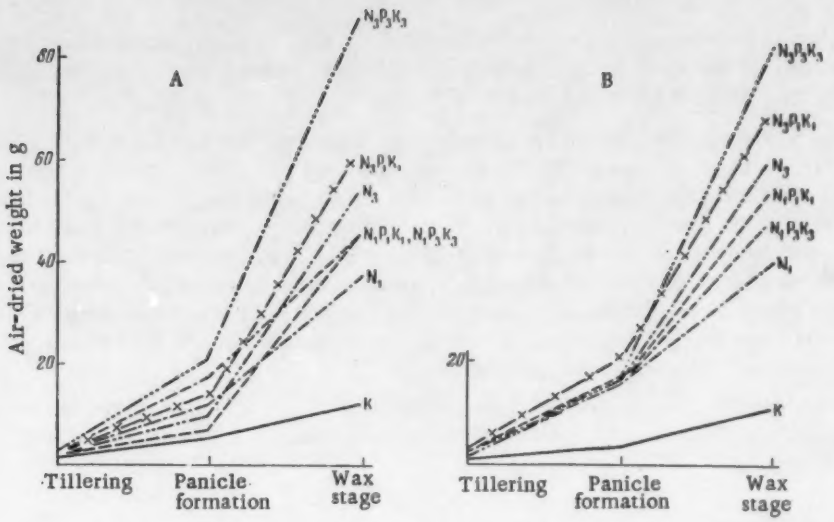

Fig. 4. Accumulation of air-dried mass of oats, variety "Zolotoi dozhd'," at various soil temperature and under various conditions of mineral nutrition. A) Soil heated; B) soil unheated.

TABLE 2

The Effect of Soil Heating and Mineral Nutrition on the Yield of Grain in Oats, Variety "Zolotoi dozhd'" (average weight per 10 plants, in g)

Experimental treatment	Grain weight in panicles			
	From the main stems		From the lateral stems	
	Heated	Unheated	Heated	Unheated
Control	5.3	4.7	—	—
N_1	14.2	14.7	4.1	1.25
$N_1P_1K_1$	14.8	19.3	6.6	4.3
$N_1P_3K_3$	14.4	17.5	6.3	3.3
N_3	13.1	20.6	11.4	7.7
$N_3P_1K_1$	15.4	18.7	14.2	12.8
$N_3P_3K_3$	16.3	17.3	21.6	15.9

mass. Thus, the dissimilar effect of fertilizer on the accumulation of aerial mass becomes clear in the heated series (and is absent in the unheated series). For example, in all treatments of mineral nutrition in the unheated series the maximum difference in the weight of the aerial mass is only 6 g (15 g in treatment N_1 and 21 g in treatment $N_3P_1K_1$), and in the heated series the difference is twice as large— 12 g (8 g in treatment N_1 and 20 g in treatment $N_3P_3K_3$). One can see from Fig. 4 that in the period prior to panicle formation the course of accumulation of dry mass was very close in all treatments in the unheated series, while in the heated series the curves were quite widely separated.

The effect of different combinations of mineral fertilizers (NPK) is shown, beginning with the shooting stage. In those treatments with P_1K_1 (independent of nitrogen levels), the yield of aerial mass and roots is higher in the unheated series than in the heated series. In the P_3K_3 treatments, the yield is lowered in the unheated series as compared to the same treatments of the heated series, and with P_1K_1 treatments of the same series. The same regularity is shown also in the rate of tillering; for example, in the P_3K_3 treatment with one dose of nitrogen (treatment IV) the number of lateral shoots in the unheated series is sharply decreased. Consequently, the same combinations of fertilizers act differently at various soil temperatures, which, in our opinion, is very important in the use of various mineral fertilizers in different climatic zones of our country.

The analysis of the final yield of total grain mass, shown in Table 2, supports S. I. Radchenko's proposal concerning the favorable action of a negative temperature gradient on plant yields.

The weight of grain in panicles of the main stems in all mineral nutrition treatments (except control) in the unheated series is greater than in heated series. Contrariwise, in the panicles of the lateral stems, the grain weight is higher in all treatments of the heated series. This is explained by the fact that the number of lateral stems is greater in the heated than in the unheated series. The increase of soil temperatures favors the formation

of a large number of lateral stems and a higher grain yield in them. However, the total weight of grain in most mineral nutrition treatments is higher in the unheated series, because of the considerable increase in the grain weight of the panicle of the main stems.

Beginning with the shooting phase, the destruction of chlorophyll was observed in the leaves of plants in the unheated series after a triple dose of all fertilizers (treatment VII). The high levels of phosphorus and potassium with normal soil temperature had an unfavorable effect on the plants. It is possible that the excess of these substances in the unheated series brought about a change in the environmental reaction, an accumulation of poisonous metabolic products, and the destruction of chlorophyll. The excess of phosphorus and potassium was successfully used by plants in the heated series, evidently without accumulation of harmful metabolic products. In the nitrogen-poor soils (control treatments), the higher yield of oats was obtained with heating of the soil. This evidently can be explained by the fact that nitrogen mobilization from nitrogen-deficient soil takes place more rapidly with heightened temperature.

The present work has been carried out under the direction of Active Member of the Academy of Pedagogical Sciences Professor F. D. Skazkin and Candidate of Biological Sciences S. I. Radchenko, to whom I should like to express my gratitude.

SUMMARY

Oats of the "Zolotoi dozhd'" variety were cultivated on heated and unheated soil; the average temperature difference of the soils during the vegetation period being 4.7°C. The aerial parts were grown under natural conditions.

Development of the plants in the warm soil was more vigorous, the period between shooting and panicle formation being especially shortened. The effect of fertilizers varied at this period depending on the temperature of the soil.

With respect to ripening, the plants developed along two different lines: those in the warmed soil ripened early, whereas those grown in the unwarmed soil ripened late. This was revealed by the higher physiological activity of the plants in the unwarmed soil (an increase in the amount of chlorophyll and soluble sugars in the leaves, more intense respiration) and a larger yield. However, large doses of phosphorus and potassium in the plants of the unwarmed soil reduced the yield of the aerial parts and grain, whereas surplus phosphorus and potassium did not produce this effect in plants grown on the warmed soil. Obviously a higher temperature of the soil leads to better assimilation and more effective use of surplus doses of phosphorus and potassium.

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THE ASSIMILATION OF NUTRITIVE ELEMENTS BY PLANTS IN THE POLAR REGION UNDER VARIOUS TEMPERATURE CONDITIONS

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For development of agriculture in the north an important, but as yet almost uninvestigated question is that of the effect of lowered soil and air temperatures on the assimilation of nutrient elements by plants [1-4].

With the aid of labelled atoms, it became possible to accelerate the study of this complex question. Experiments with the use of P^{32}, Ca^{45}, and S^{35} were carried out in 1952-1955 in the Plant Physiology Laboratory of the Kol'skii Affiliate of USSR Academy of Science. The work was begun under the direction of Professor Z. I. Zhurbitskii.

The experiments were carried out in water and sand cultures. In water culture, Knop's nutrient solution was used, with certain changes introduced by Z. I. Zhurbitskii. The changes consisted of establishing the interrelationship between nitrogen, phosphorus and potassium to correspond to the need of individual plants, and the necessary pH of the solution was obtained by a combination of mono- or di-substituted phosphates.

TABLE 1

Effect of Temperature on Assimilation of Phosphorus During Root Feeding of Plants

Temperature		Number of counts per minute		
nutrient solution	air	per 100 mg dry weight	per 100 mg of root	
			absolute values	%
Tomatoes				
21	21	11810	12710	100
21	7	9480	11228	89
7	21	5920	5774	45
7	7	8010	6496	51
Maize				
21	21	10600	3807	100
21	7	10360	3702	97
7	21	3400	1436	38
7	7	2880	1137	30
Oats				
21	21	20560	10854	100
21	7	16280	8392	77
7	21	9980	5298	49
7	7	5500	3108	29

The doses of radioactive isotopes were about 5-6 μC per 100 ml of nutrient solution for the short, 2-hour experiments, and for the prolonged experiments the activity of the solution was lowered.

In our 1953 experiments with oats, rye, and cucumbers, we noted the great effect of the temperature of the space occupied by the roots and the much smaller effect of the temperature of the surrounding air on the assimilation and translocation of phosphorus during root feeding of plants [5]. In the 1955 experiment, the effect of the temperature of the nutrient solution and of the air on the uptake of nutrient substances by tomatoes, maize, and oats was studied. The experiments were carried out in two rooms with identical windows, facing southeast. The temperature in the room in which treatments with warm air were carried out was regulated by heating. The "cold" room was not heated for the duration of the experiment. Thus, temperature conditions differing by 14° were created in the two rooms. The duration of the experiment with labelled phosphate was 5 hours. The experiments were done in duplicate. The experiment was set up with 40-day-old tomatoes, and 22-day-old maize and oats. After the experiment, the plants in each vessel were analyzed for the P^{32} content (tomatoes—3 plants per vessel, oats and corn — 6 plants per vessel). There were four variables in the experimental plan—at air temperatures of both 21° and 7° a solution temperature of both 21° and 7° was maintained. The solution

temperatures were established prior to the experiment and were then maintained throughout the duration of the experiment by means of water baths into which the vessels with plants were placed. The temperature of the water in the baths was maintained constantly at the necessary level. The results of the experiment are given in Table 1.

During root feeding of plants, the lowering of the temperature of the root-inhabited space brings about a considerably greater decrease of phosphorus uptake by plants than does the lowering of the air temperature.

Thus, the data of the experiments with tomatoes, corn, and oats confirmed the results of our experiments of 1953-1954; it may be considered established that a low temperature of the root-inhabited layer is much more greatly reflected in the uptake of nutrient substances than is the air temperature. Lowering of the air temperature has a very small effect on the uptake of phosphorus by plants.

TABLE 2

Effect of Temperature on Absorption and Translocation of Phosphorus During Foliar Feeding of Plants

Temperature		Part of the plant	Number of counts per minute		
nutrient solution	air		per plant	P^{32} translocated per 100 mg of leaf	
				In abs. values	in %
Tomatoes					
21	21	Experimental leaf	14550	4320	100
		Without exptl. leaf	259		
21	7	Experimental leaf	18600	1700	39
		Without exptl. leaf	229		
7	21	Experimental leaf	22700	3880	87
		Without exptl. leaf	543		
7	7	Experimental leaf	11700	1870	43
		Without exptl. leaf	208		
Maize					
21	21	Experimental leaf	5859	294	100
		Without exptl. leaf	119		
21	7	Experimental leaf	2032	50	17
		Without exptl. leaf	22		
7	21	Experimental leaf	2791	304	103
		Without exptl. leaf	107		
7	7	Experimental leaf	2065	44	15
		Without exptl. leaf	19		
Oats					
21	21	Experimental leaf	6320	589	100
		Without exptl. leaf	483		
21	7	Experimental leaf	2305	127	22
		Without exptl. leaf	94		
7	21	Experimental leaf	4495	2041	347
		Without exptl. leaf	1286		
7	7	Experimental leaf	1796	85	14
		Without exptl. leaf	59		

An experiment was set up simultaneously, using the same temperature treatments and the same cultures, in which the effect of temperature on the assimilation and translocation of phosphorus in the case of foliar feeding of plants was studied. The duration of the experiment was also 5 hours.

Two plants were planted in a vessel with 100 ml of nutrient solution. One leaf of each plant was immersed in a Petri dish containing 50 ml of a 0.1% solution of KH_2PO_4, which contained 30 μ C P^{32} (the temperature of the solution in which the leaf was immersed was equal to the temperature of the surrounding air). After the experiment, the leaf which was immersed in the P^{32} solution (the experimental leaf) was first cut and thoroughly washed with water; then, in order to analyze for P^{32}, the whole plant was cut into three parts—the portion above the experimental leaf, the portion below the experimental leaf, and the root. At the time of the

experiment, the tomatoes had five leaves, corn—three leaves, and oats—two leaves. The third leaf of tomatoes, the second leaf of corn and the first leaf of oats were immersed in the P^{32} solution.

With foliar feeding of plants, the lowering of the air temperature decreased assimilation and, especially, translocation of phosphorus in plants to a greater degree than did the lowering of the temperature of the root-inhabited space (Table 2).

The results of the experiments of this series, in which roots of plants were heated, may be considered reliable since they were completely confirmed by the data of the experiments carried out earlier with other cultures. The results of the experiments with foliar feeding, which were obtained for the first time, need confirmation. However, the data of the experimental results give us a basis for supposing that when the air temperature is higher than the soil temperature, the plant would be better supplied with phosphorus given by foliar feeding, since its use by a cool root system is heavily impeded. The application of foliar feeding was tested in the same year in a field experiment with tomatoes and cabbage. The use of foliar feeding with nitrogen,

TABLE 3

The Effect of Temperature on Assimilation of $C^{14}O_2$, Translocation of C^{14} Along the Plant, and its Excretion into the Nutrient Solution

Temperature		Part of the plant	Counts per minute		
nutrient solution	air		per disc	% flow from leaf	C^{14} release (roots)
		Tomatoes			
4	4	Experimental leaf	199		
		Without exptl. leaf	6	13	16
19	4	Experimental leaf	542		
		Without exptl. leaf	9	8	6
4	19	Experimental leaf	327		
		Without exptl. leaf	7	15	24
19	19	Experimental leaf	1362		
		Without exptl. leaf	61	40	1
		Corn			
4	4	Experimental leaf	1250	13	25
		Without exptl. leaf	19		
19	4	Experimental leaf	564	16	18
		Without exptl. leaf	18		
4	19	Experimental leaf	32	53	18
		Without exptl. leaf	6		
19	19	Experimental leaf	36		
		Without exptl. leaf	5	42	13

phosphorus and potassium in our experiments increased the additional growth in dry weight of plants, and also increased absorption of nitrogen, phosphorus, and potassium by plants under the low temperature conditions of the experiment. In the experiments with tomatoes, the foliar feeding had a particularly good effect on the growth of plants and on the uptake of nutrient elements. The data of this experiment simultaneously indicate that the success of foliar feeding depends on the air temperature and that it should be carried out during the warm spring days.

The intensification of phosphorus feeding of plants at low temperatures may have great significance for the synthesis of organic substances, since the process of assimilation of carbon dioxide takes place also at the lowered temperatures.

Experiments were also set up to deal with the effect of air temperature and temperature of the root-inhabited space on the assimilation of carbon dioxide from the air, the translocation of assimilants along the plant, and their excretion into nutrient solutions.

The experiments were carried out in water cultures following the same plan as in the series of experiments on the effect of the air temperature and nutrient solution temperature on assimilation and translocation of phosphorus in plants, which were described above. Carbon dioxide, labelled with C^{14} was given throughout the whole

TABLE 4

Effect of Temperature on Absorption of Nutrient Elements N, P, S, Ca, K by Tomatoes, Cabbage, Oats, and Rye (assimilation of nutrient elements by plants calculated per 100 mg of roots in relative values)

Plants	Average temperature in °C	S	P^{32}	Ca^{45}	S^{35}	K
Tomatoes	7	26	54	68	71	—
	18	100	100	100	100	—
	28	114	114	118	132	—
Cabbage	7	31	57	47	49	93
	18	100	100	100	100	100
	28	71	160	119	104	—
Oats	7	34	23	49	74	—
	18	100	100	100	100	—
	28	58	115	172	150	—
Rye	7	42	50	50	62	—
	24	100	100	100	100	—

experiment to one (experimental) leaf of the plant. The experimental leaves of 2 parallel plants were placed in a glass cylindrical container, 50 cm^3 in volumn, with a concentration of 0.2% $C^{14}O_2$ and an activity of 26 μC per container. The C^{14} activity was determined after the experiment in dried and ground plant material with the aid of an end-window counter. The excretion of C^{14} through the roots into the nutrient solution was measured after the experiment by precipitating $C^{14}O_2$ with barium chloride and measuring the activity of $BaC^{14}O_3$. The experiments were set up with 25-day-old tomatoes and 30-day-old corn. The duration of the experiment was 2 hours.

No regular effect of temperature on the assimilation of $C^{14}O_2$ was noticed in our experiment (Table 3). However, a decrease in flow of assimilants was observed in the treatments with cold air and cold solution as compared with the optimal treatment in which the air and the solution were warm. The flow was also sharply decreased in the treatments with cold air and warm solution. Cooling of the nutrient solution caused a rapid excretion of C^{14} into the nutrient solution.

With the aid of specially build thermovegetative equipment, we were able to create three sufficiently stable temperature levels of the root-inhabited space.

The equipment consisted of three glass houses, each 2.5 m^2 in area and 1.8 m high. The side walls of the houses consisted of doors which were kept open on warm days with no rain. The lower part of each house was a water reservoir covered with a lid with two rows of openings for vessels. In two such reservoirs an electric spiral was laid out and a particular temperature was maintained there with the aid of thermoregulators.

The temperature in the cold reservoir was maintained by cold tap water which was obtained from the mountain river with the aid of an electric pump; the temperature was regulated by controlling the inflow and outflow of water. But in the second half of the summer, when the snow stops melting on the mountains, the temperature of water in the rivers rises to 10° for a certain period, and the water temperature in the reservoir of the equipment rises to 11°.

TABLE 5

Effect of Temperature on Assimilation of Nitrogen, Phosphorus, and Potassium by Plants (concentration of nutrient elements in plants in relative values)

Plants	Temperature in °C	N	P^{32}	K
Tomatoes	11	82	37	112
	18	100	100	100
	27	104	114	77
Cabbage	11	126	89	102
	18	100	100	100
	27	94	76	102

To follow up the effect of a low temperature of the root-inhabited space on the assimilation of various nutrient elements on plants, a series of water-culture experiments was carried out at two or three temperature levels for a duration of 1.5-3 days. A part of the experiment in this series, as well as some vegetative experiments, the results of which are given later, were carried out in the thermovegetative establishment.

TABLE 6

Effect of Temperature on the Assimilation of Nitrogen by Plants

Plants	Temp. in °C	% of N per absolutely dry weight			Ratio of protein to nonprotein
		total	protein	nonprotein	
Tomato	11	4.75	3.55	1.20	2.96
	18	5.78	3.42	2.36	1.45
	27	6.00	4.14	1.89	2.18
Cabbage	11	6.22	4.68	1.54	3.04
	18	4.92	3.45	1.47	2.32
	27	4.60	3.11	1.59	1.95

In the water-culture experiments, 30-day-old plants of cabbage and tomato and 13-day-old plants of oats and rye were used. The experiments for the study of the assimilation of phosphorus, calcium, sulfur, nitrogen and potassium by these plants were carried out under completely similar conditions. Nitrogen, as a nitrate, was determined colorimetrically in the nutrient solution before and after the experiment. The other nutrient elements were determined in the plants—potassium on a flame photometer, and phosphorus, calcium, and sulfur by the radioactivity of P^{32}, Ca^{54}, and S^{35}.

In Table 4 the average data of 3 experiments are given, presented as relative content of nutrient elements per unit weight of root (poassium was determined in one experiment only). The results of the experiments show that the effect of temperature on different nutrient elements is altogether different. Lowering of the temperature has least effect on assimilation of potassium by plants; the assimilation of sulfur is decreased more considerably; the effect on calcium is still greater; lowering of the temperature affects most the assimilation of phosphorus and, particularly, nitrogen. With an increase of 10° in temperature (from 18 to 28°), the assimilation of nutrient elements by plants continued to increase. Only the assimilation of nitogen by oats and cabbage decreased somewhat as compared with a temperature of 18°.

TABLE 7

Effect of Temperature on Content of Nitrogen, in the Form of NO_3 and NH_4 in the Sap (in mg)

Temperature in °C	Tomatoes		Cabbage	
	NO_3	NH_4	NO_3	NH_4
9	—	—	0	0.036
18	0.055	0.030	0.089	0.026
27	0.030	0.032	0.423	0.025

Analogous data, resulting from a long vegetative experiment (30 days) carried out in the soil cultures, are given in Table 5. The temperature level of the cold treatment in this experiment was significantly different from the previous series of short-term experiments carried out in water cultures. The average temperature of the cold experimental treatment was 4° higher, 11° instead of the 7° in the previous experimental series. This relatively heightened temperature of the cold treatment of the experiment explains the sharp change in the difference of assimilation of nitrogen and phosphorus by the plants. In this experiment, nitrogen was determined in plants by the Kjeldahl method. The accumulation of nitrogen in the plants over the duration of the experiments was measured by the difference in its content in the plants before and after the experiment.

In this experiment, attention is drawn first of all to the more greatly impeded assimilation of nitrogen and phosphorus by tomatoes. Cabbage, which is well adapted to conditions of lower temperatures, reacted quite well at 11°, and assimilated nutrient elements normally. Only assimilation of phosphorus by cabbage was somewhat impeded. Under these conditions, a retardation in phosphorus assimilation was again observed in both plants, at a time when nitrogen was easily assimilated even by such a warmth-loving plant as tomato. This phenomenon of the assimilation of nitrogen changing greatly, depending on the level of the lowered soil temperature, should necessarily be considered in the application of fertilizers. At a time when additional feeding of phosphorus to plants may be carried out at a wider range of lower temperatures, the effectiveness of additional nitrogen feedings in cold soils is limited to a considerably narrower range of lowered temperatures.

In the last experiment, nitrogen was determined in plants according to the Kjeldahl method (Table 6). Besides total nitrogen, protein nitrogen was determined, and the content of nonprotein nitrogen was calculated by difference. In spite of the fact that a certain retardation in the general accumulation of nitrogen was

observed at 11° in the warmth-loving tomato, it was nevertheless assimilated well, and the greatest part of the assimilated nitrogen went into the synthesis of protein. In V. P. Dadykin's experiment, in which the temperature of the cold root-inhabited space was from +3 to +6°, even the assimilated nitrogen remained predominantly nonprotein [1].

Prior to harvesting one of the vegetative experiments, in which the root system of plants was under different temperature conditions for 50 days (during which tomatoes were in the flowering phase, and cabbage had as yet not initiated heads) nipples with test tubes were placed on the stumps of cabbage and tomato plants to collect the sap. Nitrate and ammoniacal nitrogen were determined on the following day. It should be noted that the tomatoes of the cold treatment had not produced any sap in either this or in other experiments where collection of sap flow was carried out. The same phenomenon of the absence of exudation at low above-zero temperature was observed by Val'ter and Begacheva [6] in a number of plant species insufficiently adapted to light at low temperatures.

In Table 7 are given the data of the sap analyses. Attention is directed to the following: in tomato at 27°, i.e., under the most favorable conditions for growth and assimilation of nutrient substances, the same amount of nitrate and ammoniacal nitrogen was found in the sap; at 18° a certain predominance of nitrate nitrogen over ammoniacal was already observed. The phenomenon is also observed in cabbage to approximately the same degree—under the conditions least favorable for the life of these plants at 27°, a considerable amount of nitrate nitrogen is observed in the sap. Evidently, in such cases the normal metabolism of the root system is broken down, and nitrate nitrogen is translocated into aerial organs in the same state as it was introduced into the root system.

In conclusion I should like to express deep gratitude to Professor C. I. Zhurbitskii and Professor A. V. Sokolov for consultation in carrying out the present work.

SUMMARY

Phosphorus uptake in plants fed through the roots decreases to a much greater extent if the temperature around the roots is decreased than when the temperature of the air is lowered.

The harmful effect of low temperatures on assimilation and translocation of phosphorus was much more noticeable in the case of foliar application of nutrients than in the case of root nutrition. Hence, at low soil temperatures, nutrition by foliar application seems to be more feasible than nutrition through the roots; as in the former case, the uptake occurs more readily.

When the temperature dropped to 11-12°C the assimilation of phosphorus was impeded, whereas that of nitrogen was not. A drop in temperature from 11 to 7°C noticeably reduced nitrogen uptake in plants, but only slightly affected the uptake of phosphorus. A further drop of the temperature to 5-7°C most noticeably impeded the assimilation of nitrogen and to a somewhat lesser extent the uptake of phosphorus and least that of calcium and sulphur; at low soil temperatures potassium was the most readily assimilated of these elements.

A drop in temperature to 11°C somewhat hampered nitrogen uptake in tomato plants but did not impede protein synthesis; at the same time, the ratio of nitrogen protein to nonprotein nitrogen increased slightly.

Under temperature conditions unfavorable for plant development there was a considerable increase in the content of nitrate nitrogen in the sap. The warmth-loving tomato plant in general did not excrete sap at low temperatures. Translocation of C^{14}-labelled assimilates occurred at a lower rate when the air temperature was lowered.

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PHYSIOLOGICAL PECULIARITIES OF TOMATO AND MAIZE PLANTS HARDENED TO COLD WITH ALTERNATING TEMPERATURE

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In many districts of our country late spring and early autumn frosts and abrupt diurnal temperature fluctuations are an obstacle to the cultivation of thermophilic plants—vegetables, melons and maize. These climatic features, in spite of a sufficiently warm summer and fertile soils in a number of districts, make it impossible to obtain stable high yields of vegetables and maize. This poses to the plant producers and the physiologists the problem of studying and improving existing agricultural plants and of establishing new methods for increasing their productivity. For resolution of this problem there is necessary a thorough understanding of this or that effect on the plant which causes a change in its physiology, i.e., the further development of certain theoretical problems in plant cold-hardiness.

In connection with the above, as the basis of our investigation, we began in 1956 to study the effect of hardening seeds to cold by use of alternating temperatures.

Hardening of seeds to cold (and in general to unfavorable conditions) is based on the extreme plasticity of very young plants which are just swelling and starting to grow [1]. Grachev [2] in 1875 had already successfully cold-hardened maize seeds with low temperatures, obtaining a crop of mature seeds south of St. Petersburg. Cold-hardening has been shown in a number of studies [3-14] published in recent years. Some of these studies were devoted to the effect of constant low temperatures on plants, others to the effect of alternating temperatures. The method of hardening with varying temperatures (more effective than constant low temperatures) has been studied less than the other known methods of hardening and still does not have a theoretical basis. Strictly, hardening with varying temperature involves the effect of heat and cold on the developing seed embryo during several twenty-four hour periods. The change taking place in the metabolism of the embryo as a result of this treatment then affect its physiology during all of ontogeny. Voronova [5, 6] in northern Transuralia used varying temperatures to harden watermelons, egg plants, cucumbers, tomatoes and maize. According to her data, hardening increased cold tolerance, accelerated development, increased the number of successfully matured fruits and increased the total yield. Kodymskii [12] studied seed hardening of a large number of maize varieties using alternating temperatures for periods of differing lengths and showed that the hardening effect depends upon the variety and upon the duration of the hardening treatment. The hybrid varieties gave better results after hardening from 15-20 days: acceleration of development and increase in yield. Shutov and co-workers [10] and Makaro and co-workers [7], working with cucumbers and tomatoes, found that under optimal conditions of hardening with alternating temperatures the varieties which were responsive to hardening gave greater and earlier yields.

Gunar and Krastina [13], in greenhouse experiments with maize hardened by Voronova's method, found that hardening decreased seed germination. The growth rate of young hardened plants from seeds sown in warm soil was lower than the controls. In addition to the negative effects of hardening the authors also observed positive effects: the growth rate of hardened plants from seed sown in cold soil was greater than the controls; moreover, the hardening somewhat increased cold-hardiness. In many studies [7, 10-12] workers have noted a close dependence of the effectiveness of hardening with alternating temperatures upon both the plant variety and the duration and temperature regime of hardening. Possibly the depression in germination is related to damage to the radicle, which has been severely bent during the hardening process.

Nezgovorov and Solov'ev note that the more advanced the germination of a cucumber seed, the more quickly it is damaged by cold since the root of the germinating seed is exposed to the effect of cold first [15].

In contrast to the large number of studies of an agronomic character, Shutov [10] and Makaro [7] investigated the biochemical features of hardened plants and showed that the leaves of hardened tomato plants possessed large amounts of chlorophyll, their fruits, large amounts of sugars, organic acid, and dry matter. In addition, Makaro [7] found in hardened fruits a greater amount of vitamin C and a higher catalase and peroxidase activity.

In a number of studies by Genkel' and his co-workers [9, 16, 17] experiments were undertaken to explain certain physiological peculiarities of cold-hardiness, and, in particular, of plants hardened to cold. Genkel' and Margolina [9, 16] showed that plant resistance to low temperatures was closely correlated with protoplasmic viscosity. A low viscosity conditioned a greater plant resistance to frost.

The effect of hardening maize seeds with alternating temperatures was studied in the work of Genkel' and his co-workers [9]. Hardening increased maize seed germination, speeded growth and development at low temperature, and increased the green weight yield. After exposure of the plants to a low positive temperature (+ 9°C) the protoplasmic viscosity changed; it decreased in hardened, and increased in unhardened plants. The authors explain the cold-hardiness and rapid growth of young plants at low temperatures as a consequence of the large decrease in protoplasmic viscosity of the hardened plants, i.e., more complete adaptive reactions than in the unhardened plants.

The studies of Tumanov [18], Kursanov and Kriukova [19], Blagoveshchenskii [20], Genkel' [9, 16], Semikhatova [21], and Zholkevich [22] permit us to conclude that the degree of plant cold-hardiness varies during ontogeny both in relation to change in plant environmental conditions and in close relation to the physico-chemical nature of the protoplasm, with the activities of enzymes [16], with complex harmonic combinations of the processes of synthesis and catabolism, i.e., plant cold resistance is dependent on the peculiarities of the metabolism as a whole.

Hardening and Plant Growing Conditions

The experiments were begun in 1956 and continued in 1957 in Dalmatov, Kurgansk Region (north Transuralia). According to meterological data of the Dalmatov State Meteorological Station the weather conditions during these summers were typical for this region. During the vegetative period an abrupt shift in diurnal temperature is observed: the amplitude of variation reaches 20° (hot days—above 30°, cold nights—from + 5 to + 8°).

Thus the climatic peculiarities of Dalmatov are of great interest in studies of the physiology of hardening to cold in plants subjected to abrupt natural temperature fluctuations. In 1956 the plants were grown in the fields of the Dalmatov state variety plots, in 1957 in the fields of substation of the Ural Agricultural Institute. Hardening of the seeds was done by A. E. Voronova in agreement with her suggestions for the hardening regimen.*

The maize seeds were soaked for 48 hours, and then for several days the swollen seeds were alternately held for 12 hours at −1° and 12 hours at +18°C.

The tomato seeds were soaked for 12 hours (in sacks) and then subjected to the effect of the same alternating temperatures as was the corn.

The experimental variants differed according to the hardening period and the variety. In 1956 the maize varieties, First-of-May and Success were hardened for 15 days and tomato variety Prime 1180 for 5, 15, 20 and 37 days. In 1957 maize variety Saratov-2 and tomato variety Talalikhin were hardened 25 days. Plants which developed from dry, unsoaked seeds served as controls. Two controls were set up with maize variety Saratov-2; seeds sown dry and seeds treated the same way which had also been soaked at the time of sowing.

Cold Hardiness

The temperature conditions of northern Transuralia are frequently unfavorable and even lethal for thermophilic crops like tomatoes and maize. Our experimental plants in 1956 were subjected to a natural cold-hardiness

* We use this opportunity to express our thanks to A. E. Voronova for making the completion of this study possible.

test. June 2 had a late frost and hotbed tomatoes which had been set out on open ground were markedly damaged. The frost (−2°) damaged 20-30% of the plants and killed 5-8%. Plants which had been hardened for 37 days were damaged least of all.

A frost (−2°) on September 4 completely killed all the tomato leaves. (Harvest was completed at this time, the fruits which were sheltered were covered with frost-burn spots). Maize also subjected to this frost appeared to be more resistant but nevertheless, being a thermophilic crop, the leaves and ear sheaths in all of the plants were covered with frost-burn spots. Thus a natural temperature fall seems to be inadequate for comparing the cold-hardiness of hardened and unhardened plants: it is either mild or uniformly lethal for all plants. Later we began to use experimental refrigeration.

TABLE 1

Cold-Hardiness of Tomato Leaves (% living cells in the microscope field after freezing)

Treatments	Budding	Flowering	Fruiting
	Variety Prime 1180		
	120 min at −6°	120 min at −5°	150 min at −5°
Without hardening (dry seed)	Few	38.6	3.0
Hardened 15 days	—	65.7	29.0
Hardened 20 days	Many	—	23.5
Hardened 37 days	—	87.5	15.0
	Variety Talalikhin		
	25 min at −2°	30 min at −2°	30 min at −3°
Without hardening (dry seed)	80.6	84.4	8.9
Hardened 25 days	96.7	96.2	28.6

In the 1957 experiments one leaf was cut off of six or seven plants of each experimental variant and a horizontal slice was taken from the surface of each leaf (epidermis and the underlying parenchyma). The slices were immersed in distilled water and frozen in test tubes for 20-30 minutes at −2 to −4°C. Upon reaching room temperature the slices were stained in a solution of neutral red and transferred to a one molar sugar solution. Cell death was determined by the absence of plasmolysis. The number of living cells and the total number of cells in the field of view were counted in 28-32 places in the central part of each of the six slices.

The degree of cold-hardiness of the experimental variants compared was characterized as the percent of cells alive after freezing. In 1956 experiments leaf fragments were frozen entirely, then slices were made from them and the determination described above was made.

Results of the 1956 experiments presented in Table 1 show that leaves of tomato variety Prime 1180 which had been hardened 15, 20 and 37 days were markedly more cold-hardy than the unhardened leaves. Cold hardiness of the leaves of hardened maize varieties First-of-May and Success also exceeded that of the controls (Table 2). In 1957 experiments with tomato variety Talalikhin (Table 1) and maize Saratov-2 (Table 2) confirmed that hardening with alternating temperatures increases the cold-hardiness of the leaves of these thermophilic crops.

It should be noted that hardening of tomatoes, plants which are more cold-resistant than maize, effects a great increase in cold-hardiness. Comparative data on maize indicates that a greater increase in cold-hardiness occurs in the maize variety Saratov-2 from seeds sown for the first time at Dalmatov.

Rate of Photosynthesis

The size of the yield is chiefly a result of the work of the photosynthetic apparatus, and the rate of the photosynthetic process is central in the attention of physiologists.

TABLE 2

Cold Hardiness of Maize Leaves (% living cells in the microscope field of view after refrigeration

Treatments	Vegetative stage		Flowering	Fruiting
Variety Success				
	120 min at −3°		12 hours at +1°	
Without hardening (dry seed)	53.0	—	53.5	—
Hardened 15 days	68.0	—	58.6	—
Variety First-of-May				
Without hardening (dry seed)	56.0	—	44.0	—
Hardened 15 days	56.8	—	68.0	—
Variety Saratov-2				
	20 min at −3°	30 min at −2°	30 min at −4°	30 min at −3°
Without hardening (dry seed)	27.6	6.7	1.2	33.3
Without hardening (seeds swollen)	31.8	21.5	3.6	62.1
Hardened 25 days	38.7	52.1	29.7	94.3

We determined the photosynthetic rate of tomato variety Prime 1180 (in 1956) on the basis of dry weight increase of the leaves "half-leaf method" [23]. The photosynthetic rate in the remaining experiments was determined on the basis of leaf carbon content by the method of Borodulina et al. [24]. However, in contrast to these authors, we ascertained not only the gain in carbon but also the loss of carbon from the leaves, i.e., we determined the true photosynthesis. From one leaf-half a uniform leaf section was taken for determination of the initial leaf carbon content (a) and an equal area was taken from the other half of the same leaf at the end of exposure (b). A black opaque paper cover was fitted onto another such leaf-half after a sample section was taken from this half for determination of the initial carbon content (c), then a leaf sample section was taken from this darkened leaf-half for determination of carbon content at the end of exposure (d). The amount of carbon loss during the exposure period was found by the difference (c − d) and the amount of carbon accumulated by the difference (b − a). The true photosynthesis of maize was characterized in our experiments by the amount of carbon obtained as a sum (b − a) + (c − d) and is expressed in mg of carbon per 100 cm^2 of leaf surface.

In 1956 experiments with tomato variety Prime 1180 were begun on July 2 with a 12-hour day per 24 hours. The maximum temperature was +28°, minimum +12°, a 16 degree amplitude of fluctuation. The photosynthetic rate was 2-3 times greater in hardened tomatoes than in unhardened and the photosynthetic rate of plants having different periods of hardening corresponded well with the total fruit yield (Table 3).

TABLE 3

Rate of Photosynthesis and Fruit Yield of Tomato Variety Prime 1180 in 1956

Days of hardening	Photosynthesis, g dry wt/m^2	Total fruit yield	
		kg/45 m^2	% of control
Control without hardening	5.0	113.6	100
5	11.9	119.1	105
15	10.0	116.8	104
20	16.9	123.1	109
37	13.7	122.5	108

Experiments with maize varieties Success and First-of-May were conducted in July and the beginning of August after cold nights (+7° to +13°). The daytime maximum temperature reached 27°, i.e., a diurnal amplitude of about 18°. Hardened maize plants were half and half, having half of the time a higher photosynthetic rate than the unhardened (Table 4). Unfortunately, a comparison of photosynthetic rates during ontogenesis was impossible since conditions of plant illumination during the days of the experiment could not be compared because of the effect of variable cloudiness.* Hardened maize plants, having a higher photosynthetic rate, produced a greater yield of aerial parts.

* Sharp fluctuations in illumination are characteristic for Dalmatov.

TABLE 4

Photosynthetic Rate and Yield of Aerial Parts of Maize

Treatments	photosynthesis mg carbon/100 cm^2 leaf sfc/6 hr					Dry wt. aerial parts (30 plants)	
	vegetative stage			pollinating stage		in kg	% of control
	7/19	7/23	7/25	8/4	8/8		
Variety Success							
Control (dry seeds)	9.6	—	3.8	3.9	—	20.8	100
Hardened 15 days	18.4	—	6.3	10.8	—	25.9	119.7
Variety First-of-May							
Control (dry seeds)	—	1.1	—	—	3.7	16.7	100
Hardened 15 days	—	2.3	—	—	12.3	17.4	104

The determination of photosynthesis in 1957 was made according to the method described above, but in the titration we used the new indicator—phenylanthranilic acid—proposed by Simakov [25] instead of the diphenylamine used in the method of Borodulina.

Data obtained in 1956 in determination of the rate of photosynthesis was confirmed in 1957 in new varieties: the rate of photosynthesis in hardened plants of tomato variety Talalikhin (Table 5) and Saratov maize (Table 6) were greater than in the controls. On the basis of hardened plant yield these varieties excelled the unhardened (Tables 5 and 6).

According to data in the literature, comparison of the rate of photosynthesis of different plants on the basis of their cold-hardiness shows that after exposure to lowered temperatures thermophilic plants are not able to photosynthesize, and re-establish this capability much more slowly than do cold-hardy forms [22, 26-28]. With strong cooling of thermophilic plants the chlorophyll becomes easier to extract, evidently because of a disruption in its linkages with protein; the total amount of protein in this event decreases. Suppression of photosynthesis as a result of cooling is probably a result of damage to protoplasmic structures, in particular to the chloroplasts [16].

Our determinations of the rate of photosynthesis of tomatoes and maize, obtained under the conditions of northern Transuralia permit the conclusion that plants which became more cold-hardy from hardening of seeds with alternating temperatures have a higher rate of photosynthesis after a sudden nocturnal temperature fall than less resistant, unhardened plants.

TABLE 5

Rate of Photosynthesis and Yield of Talalikhin Tomato Fruits in 1957

Treatments	Photosynthesis mg carbon/100 cm^2/12 hours		Total fruit yield	
	Vegetative stage	Fruiting	kg/45 m^2	% of control
Control (dry seeds)	52.5	26.3	103.1	100
Hardened 25 days	96.6	60.0	127.9	124

TABLE 6

Rate of Photosynthesis and Yield of Aerial Parts of Saratov-2 Maize in 1957

Treatments	Photosynthesis mg carbon/100 cm^2/12 hours		Dry weight aerial parts (25 plants)	
	Vegetative stage	Tassel pollenating	kg	% of control
Unhardened	67.5	0	18.2	100
(dry seeds)	32.6	15.1	17.5	96
Hardened 25 days	69.6	21.8	24.2	133

Rate of Respiration

The hardening of seeds with alternating temperatures changes the character of the physiological reactions of thermophilic plants to a change in the external enviroment. Evidently, the greater resistance of cells of

TABLE 7

Leaf Respiration Rate of Tomato Variety Talalikhin

Treatment	Bud stage			Flowering (fruits green)			Fruiting					
							fruits green			fruits brown		
	8	14	19	8	14	19	8	13	18	8	13	18
Unhardened	183.9	32.7	49.9	11.7	17.5	7.8	30.7	17.6	21.1	10.7	43.5	21.5
Hardened 25 days	160.4	40.9	21.5	14.9	36.8	27.6	56.8	24.2	24.6	40.8	42.8	28.6

Note: The top row of figures signifies the time of day (time of the determination).

hardened plants to lower temperatures and the high rate of photosynthesis with the abruptly fluctuating temperatures observed in our experiments were also an integrated expression of these relatively more "regular" reactions. It would be of interest to study in hardened plants the rate of respiration, an important physiological process which assures the plant of energy for subordinate vital processes and of highly active substances which participate in cellular metabolism.

The respiration rate of leaves in our experiments was determined by the Boysen-Jensen method as modified by S. V. Tageeva. In the respiration determination leaf discs were place in glasses above a solution of barium hydroxide and held at 12° or 25° in a light-proof box for an hour.

The respiration rate of tomato variety Talalikhin was determined three times per day during ontogeny according to the stage of development. The leaf respiration rate of hardened plants was higher in a majority of cases than in unhardened plants (Table 7).

Experiments on the respiration intensity of three maize varieties were conducted during the vegetative period according to the stage of development at a temperature of 23 to 25° and on maize variety Success and First-of-May in addition at a temperature of 12 to 15°. It seemed that the ratio of respiration rate of hardened and unhardened plants changed in maize during the course of ontogenesis: during the vegetative stage respiration was more rapid in the hardened plants but after transition to flowering it was more rapid in the controls. In maize variety Success (Table 8) this change in the ratio of respiration rates occurred during the pollen-shedding stage, in the First-of-May variety (Table 8) and Saratov-2 (Table 9) during the appearance of the stigma. A regular change in the ratio of respiration rate of hardened and unhardened control plants during ontogeny was confirmed in the experiments of both years and in all three maize varieties.

TABLE 8

Respiration Rate of Maize Leaves at Various Temperatures (mg CO_2/100 g dry wt/hr, 1956 experiment)

Treatments	Vegetative stage				Flowering					
	7/6 at		7/22 at		7/1 at		7/6 at		7/7 at	
	24°	14°	23°	12°	25°	12°	24°	12°	25°	12°
	Variety Success									
Control (dry seeds)	35.1	79.4	14.2	28.3	—	—	36.8	24.6	—	—
Hardened 15 days	48.6	96.5	4.2	18.8	—	—	31.5	16.5	—	—
	Variety First-of-May									
Control (dry seeds)	19.4	63.9	—	—	56.3	17.2	—	—	27.0	12.5
Hardened 15 days	37.6	70.8	—	—	81.2	47.2	—	—	15.9	3 .7

TABLE 9

Leaf Respiration Rate of Maize Variety Saratov-2
(mg CO_2/100 g dry wt/hr

Treatments	Vegetative stage			Flowering	
	6/7	6/19	6/2	7/16	7/30
Control					
Dry seeds	17.2	26.0	6.2	67.8	39.1
Swollen seeds	23.0	40.0	12.0	77.5	79.2
Hardened 25 days	57.2	41.3	4.3	57.9	27.3

It is recognized that plants with great vitality, and active processes of growth and reproduction also have a high respiration rate. The hardened plants in our experiments developed a greater accumulation of green matter (maize) or gave a more fruit (tomatoes). This corresponds in hardened plants with both a greater rate of photosynthesis and respiration in tomato variety Talalikhin (Table 7) and young maize plants (Tables 8 and 9).

There remain the following two groups of facts which do not correspond to the pattern which has been established concerning the greater respiration rate of hardened plants, i.e, that the temperature coefficient of plant respiration is usually greater than unity, and the respiration rate decreases with a fall in temperature.

In three experiments with maize variety Success and First-of-May (July 6 and 21, 1956, Table 8) we observed that the respiration rate at 14° was notably higher than at 24°. Analogous data are presented in the work of Sukhorukov and Cherepanova [30] where the respiration of peas at +2° was more rapid than at +22°. Respiration intensity is determined by the speed of many complex chemical reactions which go in various directions at a given temperature. Dzheims [31] notes that the ratio of the directions of the reactions with a change of temperature may be such that in some cases respiration at temperature T° will be greater than at T + 10°. However, it ought to be emphasized, which he did not do, , that in a living organism a change in the speed and direction of reactions with a temperature change depends upon the nature of the organism and its physiological status. It is possible that the case where the temperature coefficient of respiration is less than unity attests to a certain disturbance of the respiration, perhaps of the same order as the respiratory disturbance described by Semikhatova [21], where an abrupt temperature fluctuation under conditions of the high Pamir mountains more abruptly disturbed plant respiration, the less adapted the plant was to the high mountains. In our experiments described above, when respiration was determined at two temperatures, in cases where the temperature coefficient of respiration was less than unity the sudden rise in respiration with a temperature decrease was less abrupt in hardened plants than in unhardened. In the other determinations the thermal coefficient of respiration was greater than unity and higher in hardened plants than in unhardened.

TABLE 10

Leaf Respiration of Tomato Variety Prime 1180 at Various Temperatures During the Flowering Stage
(mg CO_2/100 g dry wt/hr 1956 experiment)

Hardening period in days	6/29	6/4	
	27°	25°	10°
Control	55.5	41.4	39.2
15	26.8	30.6	14.5
30	43.0	30.5	13.5

The other group of facts—a lower respiration of hardened plants in comparison to unhardened—was observed sometimes in tomatoes (Table 10) and in maize of all the experimental varieties at the end of the vegetative stage and at the beginning of flowering (Table 8 and 9). The ratio between rate of synthetic processes and the consumption of energy resources of plants by respiration may under certain conditions be unfavorable. In such cases a higher respiratory rate with a retardation of synthetic processes becomes inefficient. In his book Sabinin [29] describes a case of inefficient respiration in hydrophytes: with low illumination the respiratory rate became greater than photosynthesis; under these conditions only forms with a low respiration rate at the given temperature could survive. In comparing the respiration of thermophilic and cold-resistant plants Zholkevich [22] considered a more abrupt fall in respiratory rate with cooling as an expedient adaptative reaction which prevents excessive consumption of plastic materials. Dzheims [31] pointed out that the respiration of mature plants may occur "to a marked degree without purpose."

So the low respiratory rate we observed during the flowering stage or preparatory to it and the high temperature coefficient of respiration of hardened plants may be considered a means of good energy balance. In the hardened plants in our experiments with a high level of synthetic processes (more intensive photosynthesis) during the reproductive stage the ratio synthesis/catalysis is probably much greater than in the unhardened plants which are photosynthesizing more weakly and respiring more strongly. The results of our experiments and also data in the literature on the physiology of cold-resistant plant forms and plants hardened to cold lead us to conclude that plant cold resistance is linked to the nature of physiological reactions of the plant caused by changes in the external environment. First of all, physiological features of the protoplasm are altered by a decrease in the temperature of the ambient environment. Changes in the structure of the protoplasm alter the properties of the enzymes and the direction of biochemical processes which they catalyze. A harmonious reorganization of the metabolism and an "economical consumption" of energy permits cold-resistant plants not only to survive a decrease in temperature but also to carry on intensive growth and fruiting.

SUMMARY

1. Hardening the seeds of the thermophilic plants tomatoes and maize increased their cold resistance under the conditions of northern Transuralia where diurnal temperature fluctuations are great.

2. Hardening greatly increased the rate of photosynthesis and the yield of tomatoes and the aerial portions of maize.

3. The respiratory rate changed in hardened plants during ontogeny and with abrupt temperature fluctuations, apparently more suitably than in unhardened plants.

4. The peculiarities in photosynthesis and respiration observed in hardened tomato and maize plants are one of the manifestations of a harmonious change in the metabolic pattern which in turn leads to a greater cold resistance and to an increase in crop yield of plants grown from seeds hardened by keeping them for 12 hours alternately at −1°C and +18°C. In conclusion, we express our deep appreciation for the scientific direction of Professor P. A. Genkel.'

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THE WATER REGIME OF GERMINATING RED CLOVER SEEDS

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The water regime of vegetating plants in relation to the problems of drought resistance and irrigation has attracted and is attracting the attention of many investigators. It has been studied in detail and elucidated by a number of thorough monographs [1-5 and others]. The water regime of seeds sown in the field and of seedlings during the initial days of life have been investigated less. The water regime to a marked degree determines the future destiny of a seedling, its growth and the yield of the sowing at the end of the season. This shows the importance of the problem of devising effective methods for increasing the field germination of seeds and the creation of an urgently needed abundant grass system of field management.

Of especially great importance is the investigation of the water regime during germination of small-sized seeds and the seedlings of perennial herbaceous grasses and clover, the field germination of which is excessively low. According to data from the All-Union Forage Institute [6] the field germination of red clover seed, depending on the quality of sown material, is 57-65% of laboratory germination which, furthermore, is often not very high [7]. Even with use of first-class clover seed according to GOST only 60% of the sown seed germinates in the field. Use of second-or third-class seed results in germination of, respectively, 53 and 40% of the seed sown. A low field germination makes it necessary to increase the sowing rate which leads to a significant expenditure of seed, of which there is a continual critical shortage. The increase in field germination of seed, clover in particular, is therefore a real problem.

One of the causes for poor germination of clover seed in the field is the fluctuating moisture regime of the surface layers of soil (0-2, 0-3 cm) in which the seeds of this crop are mixed during sowing. The surface layer of soil readily dries out and usually has a normal moisture during rainy weather or for a short period after precipitation falls. Of great importance under these conditions is the ability of seeds to quickly imbibe the water necessary for germination and to reach complete saturation in a short time. How the seed of red clover behaves in this respect is quite poorly known since data presented in the literature [6, 7] on water absorption by clover seeds cite only long periods of time (6-8-12 or more hours) which does not give a sufficiently clear idea of the speed with which seeds are able to imbibe water under favorable moisture conditions. The absence of clarification on this question in the literature stimulated us to undertake this study.

We set up several laboratory experiments as follows: three-to four-hundred-milligram portions of superior seed of red clover variety Krasnoufimskii 523 were weighed out on a torsion balance and submerged in water. A small portion of this seed sample was more convenient for the work. With a large amount of seed spread on the bottom of a dish in a thick layer it was difficult to achieve equal water imbibition by all of the seeds taken for experiment. After a prescribed period of time the seeds were removed from the water, quickly dried on a mat of filter paper and weighed. The seeds were again submerged in water until the next weighing. After complete swelling which was determined by the absence of weight gain between two successive weighings the hard seeds were separated from each sample and their weight subtracted from the initial weight. Five parallel determinations were made in all studies. Calculation of swelling ability was made on an absolute dry weight basis, i.e., with hard seed excluded. As a result of such a method of calculation a somewhat greater amount of water imbibed by clover seeds was obtained than is usually shown in the literature.

The determinations showed that water uptake by clover seeds begins immediately after submergence and may be already detectable in 10-15 minutes (Table 1).

TABLE 1

The Pattern of Water Imbibition by Clover Seed During a 20-Minute Period

Soaking period, min	Seed water content		Change in water content between determinations % of abs. seed dry wt.	$\frac{m}{M}$, %
	% of absolute seed dry weight	% of saturation		
0	10.5	5.9	—	—
15	14.5±1.51	8.5	+ 4.0	10.4
30	19.3±0.64	11.3	+ 4.8	3.2
45	28.4±1.44	16.7	+ 9.1	5.1
60	37.0±2.15	21.7	+ 8.6	5.8
75	40.8±2.57	24.0	+ 3.8	6.3
90	55.9±2.63	32.8	+15.1	4.7
105	67.3±2.93	39.5	+12.6	4.3
120	77.3±1.92	45.4	+10.0	2.5

However, water imbibition by the seeds as observed during the first 30 minutes was comparatively slow, reaching 4.5-4.8% after 15 minutes. Then the rate of water uptake increased, and during the period 30 to 60 minutes after soaking began, the amount of imbibed water had increased 8.6-9.1% in 15 minutes. During the second hour the amount of water imbibed in a 15-minute period increased to 10.0-15.1%. After two hours the amount of water which had been imbibed by the seeds had increased from 10.4 to 77.3% which constituted 45% of the maximum absorption. So under favorable moisture conditions a total of two hours is required for imbibition of half of the water needed for germination of red clover seeds.

In Table 2 are shown the results of determinations made of water imbibition by red clover seeds for longer time intervals. In this experiment, after two hours the seeds had imbibed only 67.6% water on a dry weight basis, compared to 77.3% imbibed in the first experiment. The difference between these two results was caused by a difference in experimental method—in the first case the seeds were removed from the water for weighing every 15 minutes and, as was evident from special observations, this led to a more rapid water uptake than that by a mixture of seeds continuously submerged in water.

Weighing swollen seeds at hourly intervals showed that rapid water imbibition by clover seeds continued for approximately five hours. During this time the water content of the seeds increased 19-29% each hour (Table 2) and after five hours had reached 134.8% of the absolute dry weight of the seeds which amounted to 79% of the maximum amount of water absorbed.

During the hours following the rate of water imbibition sharply decreased, and water content rose only

TABLE 2

The Water Imbitition Pattern of Clover Seeds During a 48-Hour Period

Soaking period, hours	Seed water content		Change in water content between determinations, % of absolute seed dry weight	$\frac{m}{M}$, %
	% of absolute seed dry weight	% of saturation		
1	38.4±1.33	22.6	—	3.5
2	67.6±1.11	39.8	29.2	1.6
3	90.2±1.82	53.0	22.6	2.0
4	115.3±3.05	67.5	25.1	2.6
5	134.8±1.99	79.0	19.5	1.5
6	139.2±1.80	82.0	4.4	1.3
7	148.6±1.10	87.0	9.4	0.7
8	156.1±2.11	91.6	7.5	1.3
15	161.9±2.35	95.3	5.1	1.4
18	166.1±1.96	97.5	4.2	1.2
24	168.3±1.00	99.0	2.2	0.6
30	170.5±2.37	100.0	2.2	1.4
48	160.8±1.37	94.0	9.7	0.8

4-9%/hour. After eight hours the amount of imbibed water reached 91.6% of saturation for which about 24-30 hours were required. Consequently, with high soil moisture clover seeds may be almost completely swollen in one night.

The data presented show that clover seeds imbibe markedly faster than do the seeds of cereals in which, according to the literature [8, 9], saturation occurs in 72-120 hours, although the larger part of the imbibed water on a seed dry weight basis is very much less (38-56%). After six hours clover seeds had imbibed to 82% of saturation while in the same time wheat seeds had absorbed 43% and seeds of maize "Horse Dent" 35%.

The ability of clover seeds to avidly imbibe water and to saturate in a short time can be considered as an evolutionary development to rapid changes in the moisture of the surface layers of soil in which clover seeds may germinate. Because of this ability seeds, although confined to the upper layers of the soil, are able to imbibe in a short time, from the spring moisture increment or later rains, enough water for germination.

Dry seeds are able to imbibe water with great force [10,11]. The suction force of seeds quickly decreases in proportion to swelling and at water saturation falls to zero. In such a representation of the changes in suction force of swelling seeds the relation of these changes to water imbibition and to soil moisture tension at any particular time for seeds in general and clover seeds in particular remains unclear. In order to understand the germination process it is important to know what the seed suction force of clover seeds is near to the saturated condition, and their ability to imbibe the final portions of water. No information of this kind for clover seed can be found in the literature. Also, a limited amount of work has been done on other plants [12, 13]. This led

TABLE 3

The Suction Force of Clover Seeds Swollen to Various Degrees

Experimental variant	Concentration sugar solution, moles	Osmotic pressure of sugar solution, atm.	Water imbibed by seeds, % of abs. dry weight
1	0	0	158.4±0.52
2	0.19	5.0	149.5±1.86
3	0.37	10.2	143.6±0.58
4	0.67	20.6	140.2±0.96
5	0.91	30.8	133.8±0.75
6	1.11	40.4	130.2±1.12
7	1.28	50.3	125.8±0.79
8	1.60	73.9	118.5±0.87
9	1.80	93.2	115.2±0.72
10	1.90	104.5	113.9±1.28
11	2.03	120.5	112.7±1.85
12	2.19	142.8	108.0±1.16
13	2.48	196.4	102.2±0.73

us to conduct a short experiment with the object of attempting to analyze the problem especially for red clover seed.

An investigation of the suction force of clover seeds swollen to different degrees was conducted by weighing 300-400-mg samples of seed on a torsion balance and submerging them in sugar solutions of various concentrations. There were five replications at each concentration. The seed to solution ratio was 1: 20. With such a ratio even at maximum possible water imbibition the solution concentration would change no more than 2-2.5%. This permitted us to neglect in the calculations a change in solution concentration resulting from water absorption by the seeds since small changes do not essentially affect the data obtained. During the period of submergence the vessels containing the seeds were periodically agitated. After 48 hours the seeds were removed from the solution, quickly rinsed three times with water, dried twice on a mat of filter paper and weighed on the same torsion balances. After this the seeds were submerged in water for 24 hours for complete swelling in order to separate the unaffected hard seeds in the sample. The weight of hard seeds was deducted from the initial sample weight. All calculations were made on the absolute dry weight of the seeds which swelled.

In order to use sugar solutions for the determinations of suction force it was necessary to be satisfied before hand that the sugar did not penetrate the seeds in significant amounts during the experiment. This investigation was conducted in the following way: a weighed sample of clover seed was flooded with water for 24 hours.

By weighing before and after swelling the amount of imbibed water was determined. Seeds prepared in this way were submerged in a 1.3 M sugar solution.

Weighing of the seeds after 2 hours showed that the suction force of the solution exceeded the water-retaining force of the swollen seeds, and their water content decreased. If the sugar solution had been able to penetrate the seeds, then, if the seeds remained in the solution, their water content should increase because of added suction force as a result of sugar penetration into the seed. Periodic weighing of the seeds over a 48-hour period showed that their water content did not increase during this time; consequently, during the experimental period the sugar did not penetrate the clover seeds in perceivable amounts, which is also shown by the following data: when, prior to submergence in the sugar solution, the clover seed moisture content was about 161.4% of the initial dry weight, after being in the sugar solution for 2, 7, 24 and 36 hours their moisture content was respectively about 146.0, 144.5, 146.2 and 147.0%. This allows us to assume that sugar can be used in short-period experiments for investigating the suction force of swollen clover seeds.

Results of the determinations on the suction force of clover seeds swollen to various degrees are given in Table 3. The values of osmotic pressure of the sugar solution shown correspond with tables in the book by H. Walter [14].

From Table 3 it is evident that clover seeds in a near to saturation state (No. 2, 3) have suction forces at least of the order of 5-10 atmospheres. It has been shown by the work of various investigators [15, 16] that even at low moistures (wilting point) the soil moisture tension reaches values of the order of 10-20 atmospheres. Thus, only in soil which is drying out can a deficiency of water be tested, and that only at the end of swelling.

For a more complete understanding of the water supply of sown clover seeds, not only the relative values which indicate the amount of imbibed water in relation to seed weight are important, but also the absolute amount of water in the seeds at various stages in their swelling. This offers the opportunity to determine the

TABLE 4

Seed Water Content at Various Suction Forces

Experimental variant	Water content in total weight of moist seeds, %	Seed suction force, atm.	Water content per seed, %	Relative amount of water per seed	Experimental variant	Water content in total weight of moist seeds, %	Seed suction force, atm.	Water content per seed, %	Relative amount of water per seed
1	61.5	0	2.38	100.0	8	54.2	73.9	1.78	74.8
2	60.0	5.0	2.24	94.0	9	53.7	93.2	1.73	72.6
3	59.0	10.2	2.15	90.5	10	53.2	104.5	1.71	71.8
4	58.4	20.4	2.10	88.2	11	53.0	120.5	1.69	71.0
5	57.2	30.2	2.01	84.5	12	52.0	142.8	1.62	68.2
6	56.5	40.4	1.95	82.0	13	50.6	196.4	1.53	64.4
7	55.7	50.3	1.89	79.5					

actual amount of water deficiency. It appears to be very small. For example, one clover seed at complete saturation (assuming that on the average it weighs 1.5 mg absolute dry weight) contains a total of 2.38 mg of water. With 12% moisture in an air-dry seed on a dry weight basis every seed has about 0.2 mg of water. Every little seed must imbibe about 2.1-2.2 mg of water for full saturation (Table 4).

It is often indicated in the literature that seeds of leguminous plants require a large amount of water for germination. Such a situation is actually found when the amount of water imbibed by the seeds is related to seed weight. In the case of clover values obtained were of the order of 150-170%. Such figures seem especially large in comparison with water imbibition by grass seeds which reach saturation after imbibing 46-56% of their weight in water. The germination of clover seeds at a normal sowing rate of 15 kg/hectare requires a total of 27-30 kg of water for germination.

It is interesting to note that large changes in seed suction force occur as a result of insignificant changes in the relative and absolute water content. For example, a decrease of 10 atmospheres in the suction force (from 40.4-30.2) occurs with a total change in the water content of moist seeds from 56.5-57.2%, i.e., 0.7% or 0.06 mg. A water deficiency per seed of 0.28 mg or 11.8% of the amount of water imbibed by saturation corresponds to a 20-atmosphere suction force in the clover seed. A deficit from saturation of 0.23 mg per seed corresponds to a suction force of 10 atmospheres. Seeds sown on a one-hectare area in such a condition must imbibe 2.5-3.0 kg of water to reach saturation. Thus, only at wilting percentage on certain soils are clover seeds unable to imbibe the final amounts of water needed for complete swelling.

Despite their great ability to imbibe water, seeds at a low soil moisture content swell slowly. In such an event a situation is created for seeds which has been recorded in the literature in connection with water absorption by roots, where water flux into the plant is insufficient although the root suction force exceeds the soil moisture tension. Having a solution to this problem for roots, it can be assumed that a delay in the swelling of seeds at low soil moisture is not a result of the absence of available water for the seed but its insufficient mobility. This leads to the fact that water around the seed after the end of swelling simply is not that which was absorbed in the layer adjacent. This is an opportunity to advance the hypothesis introduced by N. A. Maksimov on the process of seed swelling, namely, "clarification of the relation between plant growth and soil moisture must be sought "not in soil resistance to the removal of water from each of its particles but in its cessation or, at any rate, in the extreme lag in water movement from one particle to another" [17]. This is probably involved in the cause of slower seed germination at low soil moisture than with a good moisture supply.

For clover seeds water mobility has a greater importance in that imbibed water does not go through different parts of the seed surface equally; it easily penetrates a comparatively small area around the hilum [18]. In our experiments clover seeds which were placed flat side down in moist soil had imbibed 113% of the initial weight in water in 24 hours. In that same time seeds with the hilum sealed up with vasoline imbibed only 12.9% water.

TABLE 5

The Growth and Water Content of Germinating Clover at Various Osmotic Pressures of External Solution

Sugar concentration, moles	Solution osmotic pressure, atm.	% emergence	Length of radicle		Length of cotyledonary node		% water, dry weight basis		The amount of water per 1000 shoots, in g
			in mm	in %	in mm	in %	emerged	unemerged	
0	0	100	31.6 ± 0.79	100	49.3 ± 0.86	100	90.4	—	12.70
0.04	1.1	98	19.9 ± 0.87	63	46.4 ± 1.04	94	90.1	—	14.00
0.08	2.1	54	10.8 ± 1.08	33	22.4 ± 0.63	45	77.2	59.6	6.25
0.11	2.9	52	5.5 ± 0.49	17	17.9 ± 0.72	36	79.1	70.6	7.26
0.18	4.8	4	6.4 ± 0.35	21	12.5 ± 0.31	25	—	68.1	3.64
0.24	6.4	0	—	—	—	—	—	57.2	2.89
0.30	8.1	0	—	—	—	—	—	54.1	2.31

Note: The moisture of sown seeds which had begun to emerge was about 60%, the water content of 1000 such seeds was 2.53 g.

So the imbibition of water by clover seeds is retarded not only by a soil moisture deficiency or its inadequately rapid movement but also because the water must be present around a certain part of the seed—the hilum. If water absorption by roots in soils at low moisture content is limited by a lag in water movement to the roots, then water absorption by seeds is hampered more in that water must move through a small region in the whole area of the seed hilum. Seeds in which the coat is more permeable to water as a result of damage, for example, through scarification, will swell more quickly and uniformily as a result of the fact that water can penetrate the whole surface and swelling depends less on the distribution and movement of soil water. This circumstance increases the significance of the seed scarification technique since probably its effect includes not only making the germination of hard seeds possible, but scratches on the seed coat facilitate the entry of water through the entire surface of the sown seed. The biological importance of such a seed coat construction in which water

enters the seed only through the hilum is that seed germination is hampered under conditions of water deficiency when there are no provisions for the growth of the germinated seeds.

In the analysis of the seed swelling process it is unclear to what extent complete water saturation is necessary for clover seed germination. No data could be found in the literature concerning the minimum water with which clover seeds germinated seedlings.

The method proposed in 1914 by Filter [19] was used for investigation of this question. Clover seeds were sown in sand moistened with a small amount of water. After the seeds were ready to emerge from the seed coat their moisture was determined. In all cases the water content in seeds which had begun to emerge from the seed coat was somewhat more than 60% (61-63%) or 156-170% on an absolute seed dry weight basis. Therefore, germination begins when seeds are completely water-saturated. However, at saturation the radicle does not grow and the seed remains in the unemergent stage. The inflow of a new portion of water is necessary for further growth. After the appearance of the radicle further water flux into the germinated seed is possible if additional suction force can be generated since swollen cotyledons are not able to imbibe water.

In order to investigate the suction force of germinating clover seeds, some which had emerged from the seed coat were sown in glasses containing sand moistened with various amounts of sugar solutions at different concentrations. On the tenth day the emerged and unemerged seeds were extracted from the sand, counted, and their water content determined. The results are presented in Table 5.

An increase of water in emerged seeds in comparison with swollen seeds was observed in variants where the osmotic pressure of the solution did not exceed 3 atmospheres. Under these conditions the germinating seed was able to obtain the amount of water necessary for germination. However, with a solution osmotic pressure of 2-3 atmospheres the germinating seeds lagged in growth because of a water deficiency. At a pressure of 4.8 atmospheres the germinating seedlings could imbibe some additional water, but it is insufficient for the shoot to appear.

At the moment that the radicles appear a sudden change occurs in the water requirement of the young plantlets. Emergence of the seeds begins at a moisture of 60-61% of seed dry weight. An increase of water content to 63-65% is necessary for further growth of the radicle but it must exceed 70-75% for appearance of the shoot at the surface. However, at such a water content the clover shoots grow slowly. Growth of the radicle is especially retarded which leads to a weakening of the plant. The water content of the shoots must exceed 80% for good growth.

The amount of water per 1000 shoots with different growth rates is shown in the last column of Table 5. In order to begin emergence 1000 seeds must contain about 2.5 g of water. Growth is possible with a water content of 6 g but is strongly retarded. A normally growing seedling in the experiment contained 12.7-14.00 g of water. So for normal growth each seedling in addition to 2.5 g of water during the swelling process must imbibe 10-12 mg more during the first day of growth. Consequently, the period which determines whether a clover shoot appears or not is not the swelling period, when the seed can imbibe water from a quite dry soil, but the period of emergence, the appearance of the sprout, when sufficient water must enter the shoot, and the suction force of the shoot is small. Water movement in the soil and its rapid transfer to the root of the clover shoot at this time has an marked importance for the obtaining of strong shoots and the formation of good seedling root systems able to assure a plant water supply from deeper soil horizons.

SUMMARY

Clover seeds immersed in water saturate completely within 18-24 hours; 91-92% of the final amount of water is absorbed in 8 hours.

The suction pressure of swelling seeds undergoes marked changes even when small quantities of water are absorbed and the water content only slightly changes.

The suction pressure of clover seeds at termination of swelling exceeds the water-retaining capacity of the soil; the rate of water uptake by the seed depends on the rate of movement of the water in the soil. An increase of water mobility in the soil by rolling promotes swelling of the seeds.

Germination of clover seeds occurs when their water content is 60-61%. A rise in water content to 70-75% is necessary for seedling growth; for satisfactory growth it should exceed 80%.

The suction pressure of young root tips of clover does not exceed 2-3 atm; therefore the critical period for sprouting is that between commencement of germination and appearance of the sprout above the gound line.

Moisture deficiency of the soil during germination greatly impairs the growth of the root system of the seedling.

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DEFINITION OF THE PERIODS OF HIGH SENSITIVITY OF PEANUT PLANTS TO SOIL MOISTURE

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Many plants exhibit a high sensitivity to the effect of environmental conditions during the time of flower-organ formation [1-3]. In this study special attention is given to the sensitivity of peanuts to water supply during the flower formation period.

Plants of the selected variety Adyg were raised with constant and variable moisture according to the following scheme: constant moisture at 80, 60 and 35% of soil moisture capacity; moisture 35%— from sprouting to the initiation of microsporogenesis (first flowers) and then 80% to the end of the vegetative period; moisture 80% until the initiation of microsporogenesis and then 35% to the end of the vegetative period; moisture 80% to the beginning of flowering and 35% thereafter.

The water supply conditions altered the growth rhythm and plant development of the peanuts (Table 1, Fig. 1).

TABLE 1

The Effect of Constant and Variable Soil Moisture on Duration in Days of Development Phases in Peanut

Plant development phases	Soil moisture, percentage of complete water capacity during the whole vegetative period			Periodic change in soil water	
	80	60	35	80% until pollen tetrad formation began, thereafter 35%	35% until pollen tetrad formation began, thereafter 80%
Emergence to formation of floral initials	10	10	18	10	18
Emergence to beginning of pollen tetrad formation	15	17	23	15	23
Emergence to flowering	28	33	no flowing	61	30
Beginning of pollen tetrad formation to flowering	13	16	"	39	7

The period from the beginning of pollen tetrad formation is extended 150% with constant minimal soil moisture of 35% and the later growth of the floral organs is so retarded that they do not flower until the end of the vegetative period. The development of peanuts and the formation of floral organs is faster under conditions of maximal moisture.

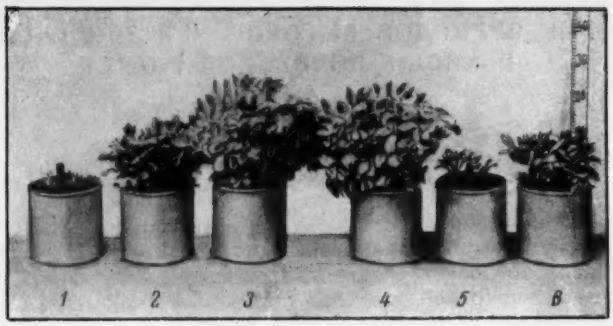

Fig. 1. 1,2,3) Constant moisture, 1) 35%; 2) 60%; 3) 80% of complete soil moisture capacity; 4) 35% from beginning of pollen tetrad formation, then 80% to end of vegetative period; 5) 80% from beginning of pollen tetrad formation, then 35% until end of vegetative period; 6) 80% until beginning of first flower formation, then 35% until end of vegetative period.

The conversion from maximal moisture (80%) to minimal (35%) after the initiation of pollen tetrads suppresses the plants severely from which they recover only after two weeks after the change in moisture regime. Under these conditions 45 days were required for the formation of floral organs even when there were present strongly developed floral initials (Table 1).

A no less marked lag in growth sets in with conversion from 80% to 35% soil moisture after flowering (formation of first flowers). In the latter case flowering is interrupted, the first flowers and buds dry up and new flowers arise after two weeks.

A periodic change in soil moisture invokes a deep disturbance in the pattern of peanut ontogeny. The effect of a water deficiency during various growth phases is not uniform.

It is evident from Table 2 that there is a direct relation between soil moisture and plant growth rate. Indications of this are the typical plant diameter, the degree of leafiness and the leaf dimensions, and also the dry weight of the aerial plant mass. In plants caught by drought during the 5-7-leaf stage after maximal moisture, an abrupt lag in growth processes occurs. With the conversion from minimal moisture (35%) to maximal (80%) after the beginning of microsporogenesis, growth processes are reactivated and, according to basic indices, the plants in a short time were as large as those which had received optimal moisture. Peanuts, as other plants which are subjected to drought, were able to utilize moisture better under conditions of copious water supply which also created conditions for intensive growth.

A large yield of vegetative plant parts was obtained with maximal moisture supply after the formation of pollen tetrads began (Table 2). Analysis of the data obtained shows the very close effect of soil water use on yield production in two groups of plants. Those which received constant maximal moisture and those grown with maximal soil moisture during the formation of flowering organs and flowering.

It is evident from comparison of the weights of roots and aerial plant parts (Table 2) that the relative root weight falls with an increase in soil moisture. This is because normal plant growth requires less development of the root system when soil moisture is adequate. Attention is attracted to the large relative root weight (in relation to the aerial parts) with continual soil drought and with periodic decreases of soil moisture to 35% after the beginning of pollen tetrad formation and appearance of the first flower. The change in the peanut root system with different soil moisture attests to its great plasticity.

Changing soil moisture also has a deep influence on the leaf structure (Table 3). Leaves formed with minimal soil moisture have a xeromorphic character: small cells, small dimensions, and thin leaf blades; the palisade tissue consisting of several rows of compact joined cells (Fig. 2 A). Under optimal soil moisture conditions the mesophyll of the leaf is increased; coarse leaves are formed with wide blades and massive veins; the palisade tissue is less developed; the leaf tissues are open (Fig. 2 B).

TABLE 2

The Change in Basic Indices of Peanut in Relation to the Soil Moisture Regime

Experimental treatment	Plant diameter, cm	Average leaf number	Leaf length, cm	Number of nuts	Weight of nuts	Dry weight	
						Aerial	Root system
Constant 80% of soil moisture capacity	30.4	44.7	11.0	100	100	100	100
Constant 35%	4.8	5.2	4.8	0	0	2.1	25
Constant 35%	21.6	23.8	8.4	64	66.6	41	71.5
80% to June 4 (beginning of pollen tetrad formation; 7 leaves), thereafter 35%	7.7	8.3	6.5	0	0	6.9	25.5
35% to June 11 (beginning of pollen tetrad formation, 5 leaves) thereafter 80%	30.6	45.8	11.1	96	106	71	101
80% to June 18 (first flower, 9 leaves), thereafter 35%	10.6	27.5	8.0	0	0	15.8	55

Note: Number and weight of nuts, dry weight of aerial plant parts, and root system are in percentage of the control.

TABLE 3

Changes in Certain Anatomical Leaf Indices of Peanut at Various Soil Moistures

Indicators	Constant soil moisture, percentage of full moisture capacity		80% to beginning of pollen tetrad formation, thereafter 35%	35% to beginning of pollen tetrad formation, thereafter 80% leaves formed with soil moisture		80% to flowering, thereafter 35%
	80%	35%		35%	80%	
Leaf thickness	8.0	5.3	5.6	5.0	6.2	5.2
Leaf thickness in the midvein region	19.5	10.0	12.0	12.0	15.0	9.0
Thickness of the layer of water-conducting motor cells	2.1	1.3	1.0	1.3	1.8	1.1

Conditions of water supply at the time that leaf formation is initiated has a substantial effect on the internal leaf structure of peanuts. A leaf formed with soil drought preserves a xeromorphic kind of structure to the end of the vegetative period in spite of subsequent excesses of soil moisture.

During the hot hours of the day the leaflets of the compound leaf are raised at an angle of 45-90° to the leaf petiole. As a consequence of this the lower, whitish side of the leaf receives a stronger effect of the sun's rays at the same time that the upper side having the stomata (Table 4) is more or less turned inward, which protects it from the effect of direct solar rays.

The ability of the leaf blade to change position increases with continual or periodic decreases of soil moisture to 35%. Conversion from the horizontal leaf position to the vertical may be considered as one of the adaptations which permits a reduction in evaporation. Anatomical investigations disclosed structural traits with the aid of which it was possible to describe changes of the peanut leaf.

In Fig. 2 in the middle of the leaf pulp are depicted coarse, thin-walled cells arranged in a kind of band along the lower side of the leaf. These are the so-called aquiferous cells known also as motor cells. In peanut the aquiferous cells lie not only beneath the epidermis, but also as separate groups found also in the midst of the mesophyll. The leaf vein of peanut is surrounded by a lining of cells in which aquiferous cells are frequently found.

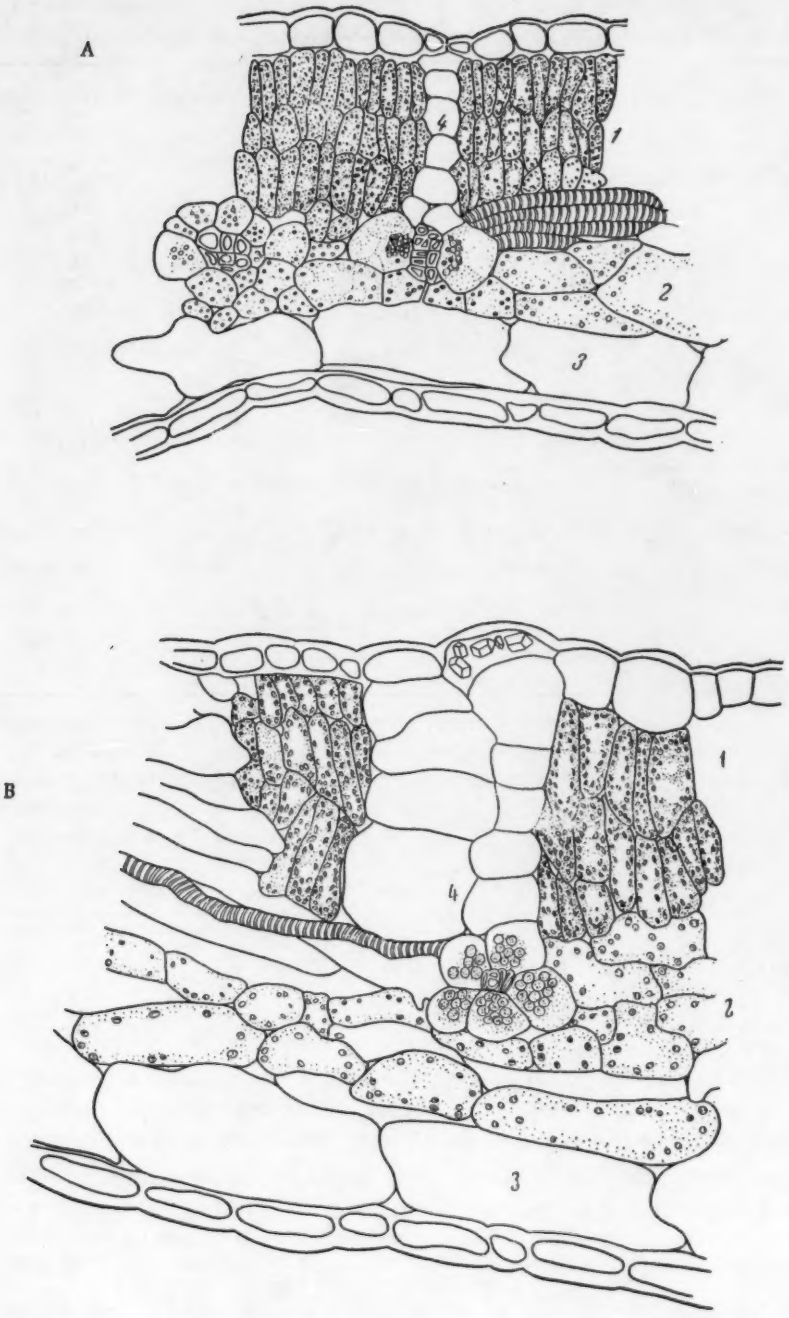

Fig. 2. Transverse section of a peanut leaf (grown with constant moisture: minimal – A (35%) and maximal – B (80%). 1) Palisade tissue; 2) spongy tissue; 3) trace of water-conducting motor cells on the lower side of the leaf; 4) water-conducting cells at the end of a leaf vein.

TABLE 4

Variation in Number of Stomata on a Unit Area of the Upper and Lower Peanut Leaf Epidermis With Various Soil Moisture Regimes (M = 50).

Number of stomata	Continual soil moisture, percentage of full moisture capacity			Soil moisture changes, percentage of full moisture capacity		
	35	60	80	35% to beginning of pollen tetrad formation, thereafter 80%: leaf formed at 80%	80% to beginning of pollen tetrad formation, therefore 35%: leaf formed at 35%	80% to beginning of flowering, thereafter 35%: leaf formed at 35%
Upper epidermis	33	28	18	20	32	33
Lower epidermis	20	22	16	14	24	19

TABLE 5

Changes in Anatomical Peanut Leaf Indices from Wilting for Two Hours (In ocular micrometer divisions)

Thickness	Fresh leaf	Wilted leaf
Leaf	7.96	6.72
Mesophyll	5.79	5.80
Aquiferous cells	2.05	1.75

The aquiferous motor cells reach a particularily strong development in Peperomia [5] and Begonia [6]. Aleksandrov [7] describes aquiferous cells in the leaf of sunflower and camel grass (Alhagi camelorum).

According to Haberlandt [8] aquiferous tissue serves as temporary water storage. Holtermann [9] ascribed to the aquiferous cells the ability to accumulate water independently of the roots (e.g., from dew). Linsbauer [10] and Warming [11] noted the presence of tannic material in the watery cell sap and considered this a protective adaptation to drought.

In the peanut leaf the aquiferous motor cells develop variously in relation to soil moisture conditions (Fig. 2). The aquiferous cells along the lower side of the leaf and near the end of the vein might be considered an indication of adaptation which permits the plant to retain the hydration and vitality of its plasma at various soil moistures. Optimal water supply conditions intensify the mesophyll of the peanut, create an optimal ratio of growth and development, and augment the yield.

Anatomical studies of the peanut leaf helped to determine the relation between positions of the leaf blade and the presence of the motor – aquiferous cells. The aquiferous, transparent cells are not only moist, transparent deflectors, which reduce the heating and drying of the mesophyll but, as shown by experiments with drying of the peanut leaf, these cells can give up their moisture to the mesophyll (Table 5).

It follows from Table 5 that the cells of the aquiferous cell layer of the peanut leaf in the turgid condition occupy a large volume. They shrink withdesiccation of the leaf. The mechanism of folding of the peanut leaf blade has been related to volume changes in the aquiferous cells distributed along a vein among the mesophyll. A decrease in the volume of the aquiferous cells because of a water loss causes a folding of the leaf blade. Therefore the aquiferous cells have also received the name motor cells (V. F. Razdorskii).

SUMMARY

The soil moisture requirement of the peanut plant varies with its growth and development;it is least from sprouting up to formation of the flower organs. Soil moisture is most profitably used for crop growth during flower formation and flowering.

The crop yield of plants abundantly supplied with soil moisture at these stages is equal to that of plants abundantly supplied with water at all stages of plant growth.

The most advantageous and rational time for watering is when the peanut plant is most sensitive to the soil water supply.

The rhythm of growth and development of the peanut depends on how the plant is supplied with water. High plasticity of the peanut is also revealed in great mesophily of the plant (increase in size and thickness of leaves, decrease in number of stomata, increase in size and number of aquiferous cells) and can be regarded as adaptive structures which provide maximum productivity. Wilting of the leaf of peanut plants is related to changes in the volume of water-conducting cells situated among the mesophyll along the veins.

The degree of development of the aquiferous cells may be useful in practical plant selection for assessment of drought resistance of different varieties of the peanut plant.

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DISRUPTION OF CARBOHYDRATE METABOLISM DURING EARLY LODGING OF BARLEY ON PEAT SOILS

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One of the characteristic features of growth and development of the grains growing in peat-bog soils is the quite rapid rate of vegetative growth. Comparable data concerning the progress of plant growth on peat and mineral soils during the same time interval have disclosed that plants growing on peat soils produce an extremely tall stem with wider and longer leaf blades than those on mineral soils. An especially rapid rate of plant growth (in barley and several other cereals) and development of vegetative organs was observed, usually during the first half of the growing period. Later the rate of increase in vegetative tissues gradually slowed down. However, the growth process continued somewhat longer than it did on mineral soils.

The abundant nitrogen supply and higher water content of the peat soil which made vigorous plant growth possible very frequently resulted in lodging. Various types of lodging during later stages of growth are described in considerable detail in the literature. The causes of this phenomenon have also been described [1-7, 8, 9, 10, 11, etc.]. But on peat soils lodging is also frequently observed during early phases of development. This lodging, however, is somewhat unusual – it is reversible. During the booting stage the grains, especially barley, are inclined toward transitory lodging. In this case, as well as frequently during later stages of growth, individual segments of the plant lodge. After a period of lodging the plants stand erect once more. Sometimes an attempt was made to attribute the cause of such lodging and recovery of plants to a temporary decrease in turgor of the cells in the stem, which was followed by a gradual recovery; this was observed most frequently under conditions of extreme changes in soil moisture from an inadequate water supply to a profuse one [2].

However, in peat-bog soils during the period of early lodging the moisture content of the soil and the air is, as a rule, sufficiently high. Consequently, the early lodging of grains on peat-bog soils must be induced by other causes.

It is more likely that during the period of rapid growth, when the plants add a considerable mass of vegetative tissue, the formation of mechanical elements in the developing stem occurs slowly, the cell walls in the ring of sclerenchyma are not thick enough and are weakly lignified.

Observations made on cross sections of stems revealed that one could detect certain special features of internal plant structure even during the early stages of growth in these plants. For example, at the beginning of the booting stage the width of the wall of a barley straw from mineral soil was greater (574 μ) than that of a barley straw from peat soil (536 μ). This same relationship was observed in the width of the cell walls in the hypodermal layer. This value was 1.88 μ in plants from a mineral soil, but 1.61 μ in plants from a peat soil. The inside diameter of the cell, essentially the vacuole, was in the reverse relationship, i.e., it was somewhat larger in plants from peat soils. When the plants were examined at the very early stage of growth – at the beginning of stem formation, the variation in internal structure was not so distinct. Depending on the degree of growth and development, these variations increased and became characteristic of the internal structure of the stem which had grown on peat soil. However such behavior was true not only of summer grains, but also of winter ones. A comparison of the anatomical structure of stems of varieties of winter wheat from peat and mineral soils quite clearly shows the marked variation in development of their supporting tissues.

The data obtained from cross sections of wheat stems show (Table 1) that the width of the stem wall and

TABLE 1

Anatomical Data Concerning the Development of the Stem in Three Varieties of Winter Wheat on Mineral and Peat Soils During the Booting Stage * (in microns)

Objects measured	Moskovskaia 24-53		Local variety		L-18-66	
	On soils					
	Mineral	Peat	Mineral	Peat	Mineral	Peat
Width of stem wall	820.7	707.5	640.2	562.6	785.7	620.8
Width of mechanical ring	55.1	50.8	64.8	62.4	73.2	62.0
External diameter of cells from the mechanical tissue in stem	17.8	23.2	19.9	20.5	—	—
Width of cell wall	5.7	4.5	6.8	4.9	7.3	5.4

* Sections were made over the second lower node.

the thickness of the mechanical ring in plants from the peat soil were notably less than in plants which had grown on mineral soil. The external diameter of the cells from the mechanical tissue of the wheat stem was greater in plants from peat soil than in those from mineral soil. However, this occurred at the expense of the increase in the cell vacuole and not because of the increase in thickness of the cell wall; under these conditions the thickness of the wall was very much less than that in plants from mineral soil. Consequently, in spite of the seemingly better external growth of the stem on peat soil, the accumulation of mechanical elements occurred at a slower rate than in plants on a mineral soil.

Very heavy dews which fall on the peat soils at night settle on the profusely developed leaf surface and become so heavy that the young stems with weakly developed mechanical tissue cannot stand up under it, and severe reversible deformation occurs.

Hence, the validity of this explanation as the cause of early lodging is confirmed, on the one hand, by anatomical data concerning the degree of mechanical tissue development in the stem under these conditions, and, on the other hand, by the fact that such transitory lodging under these conditions is usually observed in the morning.

The straightening of the lodged plants cannot be explained by the removal of the weight, i.e., evaporation of water which had settled on the leaves, since this is gone within several hours, whereas the plants return to a vertical position slowly over a period of several days. Apparently, straightening of the plants is related to the growth processes, the new formation of cellulose, and gradual strengthening of the cell wall; since this phenomenon occurs during the period of rapid plant growth it results in an increase of elasticity and rigidity of the stem.

There is evidence in the literature that the movement of the transpiration stream and the translocation of metabolites in the axial organs and the root system is disrupted during lodging [6]. However, this phenomenon has not been studied very closely, and we have found no direct experiments concerning the disruption of carbohydrate metabolism and the translocation of photosynthates as related to lodging of grains reported in the literature. Therefore, in the spring of 1955 we made some observations on the early lodging of barley, variety Viner, on the experimental plots at the Minsk Bog Experiment Station.

Our observations on the carbohydrate content of the stems and leaves of such lodged plants as compared with the distribution in some unlodged ones revealed that there was a disruption of carbohydrate metabolism during this period. The content of soluble forms of carbohydrates in these plants became quite different.

Data obtained from analyses of mono- and disaccharide content of the leaves and stems of lodged and unlodged plants during the booting stage are given in Table 2.

From the data in Table 2 it is evident that lodged and unlodged plants differ from each other in sugar content of the leaves and stems. There were 1.5 to 2 times more monosaccharides in the leaves of unlodged plants in the morning as well as during the middle of the day. An even greater variation was observed in the monosaccharide content of the stems. The stems of unlodged plants contained 2 to 3 times more monosaccharides than the stems of lodged plants.

TABLE 2

The Effect of Lodging on the Formation of Sugars in Barley Plants (in mg per 1 g dry weight)

Time of analysis	Lodged plants				Unlodged Plants			
	Leaf		Stem		Leaf		Stem	
	Monosaccharides	Disaccharides	Monosaccharides	Disaccharides	Monosaccharides	Disaccharides	Monosaccharides	Disaccharides
4:30	30.5	1.4	55	0	50	37	100	43
12:00	34.7	0	34.1	10	63	51.6	105.6	35.2

Analyses of the leaves and stems for disaccharide content also revealed a considerable variation between the lodged and unlodged plants. There were either no disaccharides, or else a very small amount, in leaves and stems of lodged plants. Whereas, in the meantime, there was a sufficiently high sugar content in the leaves and stems of unlodged plants.

Such notable variations in the content of soluble forms of carbohydrates are evidence of disruption in the course of their formation in lodged plants.

The role of glucose as one of the mobile products of photosynthesis used in the formation of materials which make the plants resistant to lodging is indisputable. Glucose is the raw material for the synthesis of cellulose. However, recently there has been more and more evidence concerning the important role of sucrose in the living processes of plants. Some investigations [13, 14, 15, and others] indicate that in the process of photosynthesis sucrose precedes the simple sugars which, apparently, appear secondarily as the result of hydrolytic dissociation. Sucrose is the main carbohydrate translocated, and its predominance in the vascular bundles is characteristic of many plants. It participates in the respiratory process where it serves as a source of energy that is closely related to the translocation of photosynthates; these become partly dissociated and increase the hexose fraction.

Therefore, in addition to the course of monosaccharide synthesis and translocation, it is essential to know the rate of sucrose synthesis and its translocation into other plant organs. It is especially important to note this if we consider that the course of the formation of mechanical elements in the stems of cereals plays an important role in peat soils.

The difference in dry weight of the leaves and stems from lodged and unlodged plants should also be noted (Table 3).

TABLE 3

Dry Weight of Lodged and Unlodged Barley Plants (in %)

Sample No.	Leaves		Stems	
	Plants			
	Lodged	Unlodged	Lodged	Unlodged
1	12.0	14.6	8.4	10.8
2	13.4	16.6	9.0	11.4
3	14.1	18.4	8.5	12.5

A determination of the dry weight of lodged barley plants showed that the leaves and stems of lodged plants clearly contain less dry material than unlodged plants. This can also be used as some evidence that the carbohydrate metabolism of lodged plants has been disrupted, since most of the dry material of plants consists of carbohydrates.

The observed variations in mono- and disaccharide content as well as those in dry weight of the lodged and unlodged plants during their early period of development can be explained to a considerable degree by variations in the condition of the light relations which appear at the time of lodging. During early lodging of the plants the leaves overlie each other quite closely; this brings about mutual shading. The noticeable unfavorable change in light relations interferes with the normal progress of photosynthesis.

Plant lodging occurs as the result of the inadequate formation of supporting elements in the stems. However, the causes of the inadequate rate of cellulose formation and development of stem structure during the very rapid accumulation of vegetative tissue on the peat soil are apparently located in the specific metabolism of the plants as a whole in these soils.

Sapozhnikov [16] disclosed that the direct products of photosynthesis can be not only carbohydrates, but also protein substances. Recently Nichiporovich with his co-workers and others [12, 17-20], using labeled atoms in a series of investigations showed that the direction in the formation of direct products of photosynthesis to either carbohydrates or proteins depends on the conditions under which photosynthesis occurs. In particular, they indicate the great significance of nitrogen supply in the soil, and also the light conditions, for the direction of the synthesis of organic compounds in plants and the relationship between the synthesis of proteins and carbohydrates. Therefore, we can conclude that one of the causes for the observed inadequate synthesis of cellulose and the weak development of supporting tissues in the stems of grains growing on peat-bog soils is the predominance of nitrogenous substances formed as direct products of photosynthesis instead of the synthesis of simple, as well as complex, carbohydrates, especially those essential for the synthesis of supporting tissues in the stem.

Whatever the causes of plant lodging during the early phases of growth might be, the lodging itself is coupled with a disruption in the course of the formation of basic structural material for the structural tissues of the stem – cellulose and especially lignin. This has a deleterious effect on the resistance of plants to lodging even during their later periods of growth. It is possible that lodging observed during the flowering stage and the milk stage begins especially in those organs of the plant which have been injured by this phenomenon during the earlier stages of growth. We observed such a coincidence, but special observations are necessary to confirm it.

SUMMARY

Abundant moisture and surplus content of easily assimilated nitrogen compounds in peat soils leads to the energetic growth of vegetative organs and often causes transitory lodging of young plants.

During the transitory lodging of barley plants in the booting stage a disruption of carbohydrate metabolism was observed. There was a reduction of mono- and disaccharides in the leaves and stems of lodging plants. The fall in disaccharide content was very noticeable. The dry weight of lodged plants was relatively smaller.

Supporting tissues in the stems of cereals on peat soil were less developed than those in plants grown on mineral soils. Evidently, when plants are abundantly supplied with nitrogen, the formation of nitrogenous substances, which are constituents of the protoplasm of the cells of rapidly growing organs, is appreciably enhanced. This takes place at the expense of the formation of mechanical parts in the stalk.

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SALT-HARDINESS AND VARIOUS METABOLIC PATHWAYS IN GLYCOPHYTES

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Among the glycophytes, i.e., the plants from non-brackish soils, there is a series of forms which are able to become adapted to some degree or other to conditions of soil salinity during the growing period. Sugar beets, cotton, quack grass, barley, and others belong to this group. In these plants the change in some of the features in the background of over-all depression is of an adaptable nature. Salt-hardiness of crops can be determined by their different biological properties: considerable resistance of the plant tissue to salts (sugar beets [1-3]), vigorous development of a root system and its distribution in the deeper horizons of the soil with fewer salts (alfalfa in the second and third year [4]), etc. It is probable that the adaptation of glycophytes to soil salts, as well as that of halophytes [5-8], is very diverse.

It is necessary to note that in the phenomenon of salt-hardiness of glycophytes, in addition to the degree of salinity of the soil the nature of the salts also plays an important role, i.e., which of the following ions are present in the given soil SO_4, Cl, or HCO_3. According to data in the literature, Cl and SO_4 ions do not have the same effect on plants. The first one inhibits but the second one stimulates the respiration of halophytes [9, 10]. The succulence of halophytes is more clearly expressed with chloride ions than with sulfate ions [10]. Cotton plants growing under conditions of chloride-sulfate salinity acquire the characteristics of xerophytes, whereas cotton plants growing under conditions of sulfate-chloride salinity acquire halophyte characteristics [11]. According to Lazko's data [12], the Cl ion induces a decrease in the rate of photosynthesis of potato, a decrease in the activity of invertase, amylase, and β-glucosidase as compared with the SO_4 ion. From this we can conclude that the adaptation of glycophytes to the salt content in the soil also varies with the kind of salts which are present.

The present investigation is concerned with a study of the effect of soil salts on several glycophytes which vary in their hardiness to salts.

The following plants were used as experimental objects: sugar beet, winter wheat, Sudan grass, alfalfa during first- and second-year growth, perennial grasses in their second year – fescue, broad-leafed barley, quack grass. The plants were all grown under irrigation in the Stalingrad Province on the Baluevskii Experimental Improvement Station. The principal soil of the fields consisted of soils transitional between light-chestnut to dark chestnut, alkaline soils containing salts of sulfates-chlorides. The degree of soil salinity under each crop is given in Table 1.

According to the data in the literature, sugar beet and perennial grasses in their second year are more salt resistant than winter wheat, one-year-old alfalfa, and Sudan grass. However, salt resistance of crops can change considerably in relation to the conditions of growth: moisture and physical properties of the soil, agricultural practises, weather conditions, etc. In order to determine the salt-hardiness of the plants in these experiments several methods were used: sprouting the seeds, microscopic method, and an evalutation of the degree of salt-hardiness by the yield of the plants.

The seeds from the various crop plants (perennial and annual grasses) were sprouted under laboratory conditions, not in salt solutions as is usually done, but in naturally brackish soil which contained different concentrations of salts.

According to the data from the experiments, the greatest salt-hardiness was demonstrated by Sudan grass

TABLE 1

Salt Content in % of Absolute Dry Weight of the Soil

Degree of salinity	Horizon	Two-year-old grasses and one-year-old alfalfa				Sudan grass				Winter wheat				Sugar beet			
		Cl	SO_4	HCO_3	solid residue	Cl	SO_4	HCO_3	solid residue	Cl	SO_4	HCO_3	solid residue	Cl	SO_4	HCO_3	solid residue
Weak salinity	A	0.009	0.013	0.043	0.170	—	—	—	—	—	—	—	—	—	—	—	—
	B_1	0.008	0.010	0.058	0.180	—	—	—	—	—	—	—	—	—	—	—	—
	B_2	0.002	0.002	0.092	0.190	—	—	—	—	—	—	—	—	—	—	—	—
	C	0.009	0.870	0.087	1.480	—	—	—	—	—	—	—	—	—	—	—	—
Medium salinity	A	0.045	0.013	0.072	0.266	0.040	0.012	0.105	0.450	0.056	0.038	0.072	0.494	0.041	0.076	0.038	0.843
	B_1	0.137	0.115	0.054	0.729	0.039	0.012	0.105	0.375	0.123	0.313	0.038	0.995	0.102	0.265	0.026	1.283
	B_2	0.144	0.282	0.056	0.794	0.166	0.265	0.375	0.750	0.231	0.323	0.048	1.265	0.161	0.282	0.033	1.371
	C	0.192	0.251	0.044	1.831	0.178	0.338	0.036	1.875	0.109	0.304	0.048	1.132	0.393	0.227	0.035	1.728
High salinity	A	0.394	0.128	0.034	1.253	0.213	0.253	0.056	2.875	0.314	0.476	0.033	1.500	0.281	0.076	0.024	1.300
	B_1	0.348	0.154	0.037	1.201	0.148	0.278	0.041	2.625	0.282	0.231	0.037	1.687	0.323	0.231	0.023	1.419
	B_2	0.288	0.333	0.050	1.463	0.176	0.313	0.048	2.125	0.233	0.306	0.040	1.875	0.612	0.307	0.033	2.904
	C	0.139	0.251	0.057	1.047	0.111	0.338	0.033	1.437	0.170	0.307	0.043	2.500	0.371	0.360	0.023	2.517

and fescue, and the least by crested wheat grass and quack grass. However, the sprouting method can be used only as preliminary indication of salt-hardiness. As Koval'skaia and Genkel' [9, 13] showed, this characteristic does not remain constant but changes in relation to the stage of plant development.

In order to check the salt-hardiness of plants during various periods of their development we used the microscopic method [14] which consisted of the following procedure: sections of leaf epidermis were immersed in a 1.0 M solution of sodium chloride and after a certain number of hours the number of living (plasmolysed) cells in the field of the microscope was counted. Using this method it was demonstrated that the salt-hardiness of grasses was greatest in the period before the formation of reproductive organs. During the transition to the reproductive stage the hardiness of the plants to salts dropped sharply. In winter wheat the greatest resistance to salts was noted during the early periods of growth. This might have been due, on the one hand, to their going through the vernalization stage, as reported by Sergeev [15], or on the other hand, to factors of the surrounding medium. As we know, the concentration of salts in the soil is always greater in the fall than in the spring when there is always a considerable decrease because of washing by winter-spring precipitation. Winter wheat plants growing at a higher salt concentration during the first phases of growth also developed a great hardiness to salts. In the spring the salt-hardiness of winter wheat increased from the tillering stage to the booting stage, and dropped sharply again during the period of reproductive organ formation during the heading phase, and especially during flowering.

Thus, using the microscope method we were able to show the dependence of salt-hardiness in the plant to the conditions of the external media, and to the condition of the plant itself (phase of development). However, the microscopic method indicates the resistance of only the integumentary tissue – the epidermis – to the osmotic and toxic effect of salts. Salt-hardiness of the plants, naturally, is determined not only by the resistance of the integumentary tissue, but by the biology and physiology of the entire organism as a whole.

Therefore, the most objective criteria of the salt-hardiness of plants is their growth and reproduction under brackish conditions. In this particular case instead of using the absolute volume of yield as an indicator of salt-hardiness we used the degree of its decrease during the change from less brackish soils to more brackish ones. We should also note that the

TABLE 2

Size and Number of Cells in the Upper Epidermis of the Leaves (middle layer) of Sudan Grass and Alfalfa at Various Soil Salinities

Salinity	Sudan grass			Alfalfa		
	Leaf area in cm^2	Size of cells in μ^2	Number of cells in the entire leaf blade in thousands	Leaf area in cm^2	Size of cells in μ^2	Number of cells on the entire leaf blade in thousands
Medium	105.7	915	11550	7.3	865	845
High	64.6	799	8200	3.1	700	443

relative decrease in yield indicates the degree of salt-hardiness of the crop plants regardless of the means by which this hardiness was achieved. The productivity of all the crops decreased as the brackishness of the soil increased. But the degree of decrease was not the same. Sugar beet exhibited the smallest decrease in yield (368 centner/hectare of roots at medium salinity, and 330 centner/hectare at high salinity), then two-year-old alfalfa, quack grass, and fescue (dry weight of vegetative tissue corresponded as follows: 16.7 and 8.9; 14.8 and 7.8; 2.3 and 1.14 centner /hectare); the greatest occurred in winter wheat (20.6 and 2.9 centner /hectare of grain); fescue (9.8 and 3.4 centner /hectare grain) and Sudan grass (91.0 and 31.3 centner/hectare grain) occupied an intermediate position. The decrease in yield occurred primarily because of the inhibition of the growth processes: the growth of the stem, roots and leaves was depressed; bushiness and accumulation of dry weight decreased; the size of the heads (inflorescences) and the number of heads decreased; and the absolute weight of the seeds was less.

The change in anatomical structure of glycophytes as the result of increased soil brackishness was exhibited by the decrease in the size and number of the cells on the entire leaf blade. Hence, in glycophytes, in contrast to the halophytes, the reduction in size of the organs as the result of the effect of salts occurs because of the decline in cell division as well as cell enlargement (Table 2). This relationship was also observed by Brovtsyna [16, 17] in cotton.

In addition to the depression of growth, a deviation from the normal course of plant development was also observed under conditions of extremely high soil salinity; the growth of vegetative organs was slowed down, and reproductive development was accelerated.

The inhibition of growth processes and the change in plant development occurred as the result of metabolic changes; in our experiments the rate of respiration was used primarily as an indicator of this change.

TABLE 3

The Effect of Soil Salinity on the Respiration Rate of Glycophytes (mg CO_2 per 100 g dry weight per hour)

Plants	Vegetative phase			Reproductive phase	
	salinity				
	medium	high	low	medium	high
Sugar beet	27.9	35.5	—	—	—
Winter wheat	135.0	100.0	—	180.0	150.0
Two-year-old grasses:					
Alfalfa	103.0	121.7	80.0	110.0	130.0
Fescue	52.3	77.4	60.0	90.0	115.0
Quack grass	91.6	98.9	95.0	130.0	110.0
Barley grass	—	—	60.0	120.0	155.0
One-year alfalfa	43.3	26.6	—	33.0	15.8
Sudan grass	190.0	240.0	—	155.0	173.3

Concerning the problem of plant respiration as well as that of reproduction we were interested not in the absolute value of these processes in different plants under brackish conditions, but rather in the degree and direction of change in respiration during the transition from less brackish soils to more brackish ones. The data obtained concerning this are given in Table 3.

As we see from Table 3, under the conditions of our experiments a decrease in the respiration of some of the glycophytes was noted as the salt content of the soil increased; in others there was an increase. Alfalfa, in the first year of growth, and winter wheat belong to the first group; sugar beet, two-year-old perennial grasses, and Sudan grass belong to the second group. Such a heterogeneous reaction among the glycophytes can be explained by a series of factors.

In this respect, some dependence on the degree of salt-hardiness can be established for some of the glycophytes. For example, the rate of respiration will decrease in less salt-resistant plants and increase in more salt-resistant ones. The clearest illustration of this principle is, on the one hand, the change in respiration of sugar beet under the influence of increasing soil salinity, and, on the other hand, that of winter wheat. Apparently, an increase in respiration, in this case, can be viewed as an active reaction of more salt-resistant forms of plants at high salt concentrations of the soil, or as an active opposition of the organism to an unfavorable factor of the environment.

TABLE 4

Effect of Soil Salinity on Catalase Activity (in ml $KMnO_4$ per 1 g fresh weight)

Salinity	Winter wheat	Fescue	Quack grass	Barley grass
Medium	16.9	13.0	12.3	10.6
High	31.1	19.0	15.4	14.0

Together with the special biological features of the plants, the nature of the salinity of the soil may also have played an important role in this case. The increase in the rate of respiration of Sudan grass as the degree of soil salinity was increased serves as an example. An analysis of the specific chemical composition of the soil below the Sudan grass (Table 1) reveals that under conditions of high salinity the sulfate salts exerted a greater effect on the plants than the chloride salts to the degree that the SO_4 ion was predominant over the Cl ion in the soil. Apparently the increase in respiration of Sudan grass is associated with the dominant effect of the sulfate ion during the transition from medium salinity to high salinity. The inverse reaction to salinity by one-and two-year-old alfalfa is also interesting. These two plants differ from each other in the structure of their root system and the distribution of its actively working surface in the soil. During the first year the root system of alfalfa was located in the upper layers, where the greater amount of chloride salts were concentrated in the given experiments (Table 1). Their toxic effect on the young plants was especially great; this resulted in a sharp depression of metabolism and a decrease in respiration rate. During the second year of growth the root system of alfalfa penetrated into the lower horizons of the soil where the salinity was caused by the sulfates. As a result, as the soil salinity increased the mature alfalfa plants experienced a greater effect of the sulfate ion than that of the chloride ion. And finally, it is also possible that a different reaction of the plants also occurred: there is some increase in the rate of respiration as the degree of soil salinity increases.

The activity of oxidizing enzymes is firmly connected with respiration. A determination of polyphenoloxidase and peroxidase activity revealed that a change in their activity as the result of soil salinity did not always agree with a change in the rate of respiration induced by this factor. Sometimes this change was opposite to the change observed for respiration. An increase in catalase activity as the salt content of the soil increased was observed in all the plants studied regardless of their special biological features (Table 4).

Catalase which detoxifies the peroxide compounds that appear during the life processes of the organism performs not only an oxidizing function, but also a protective one [18, 19]. Halophytes, plants well adapted to the presence of salts, are characterized by an extremely low activity of this enzyme [20]. In glycophytes a brackish condition which induces a disruption in metabolism apparently results in an increase of toxic products of the peroxide type. Hence, the increase in catalase activity can be considered as a factor which counteracts the effect of high concentrations of salts on plants to some degree.

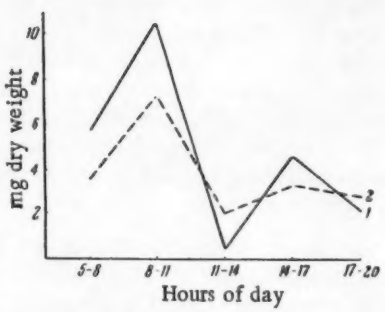
A study of photosynthesis in Sudan grass revealed that an increase in the salt content of the soil resulted in an increase in the rate of photosynthesis in this plant. On a cloudy day the rate of dry weight accumulation of Sudan grass in a soil with medium salt concentration consisted of 57.6 mg per 1 dm^2 (average of 10

determinations) of leaf area in 13 hours, in a soil of high salt concentration it was 66.3 mg; on a clear day the corresponding values for the rate of dry weight accumulation were 44.4 mg and 61.3 mg. There was no difference observed between the translocation of photosynthates in plants from soils with a medium salt concentration and those with a high one. Therefore, in this particular case the higher rates of dry weight accumulation in Sudan grass from a highly brackish soil may be related to the increase in the rate of photosynthesis. However, the increase in the rate of photosynthesis did not result in an increase of plant yield. Owing to the inhibition of growth processes in Sudan grass on highly brackish soils the size of the actively photosynthesizing surface was reduced, and consequently the total photosynthetic productivity was reduced. In accord with data in the literature [21], the total productivity and not the rate of photosynthesis determines plant yield.

TABLE 5

The Effect of Presowing Seed Treatment on the Quantity and Quality of Winter Wheat and Sugar Beet Yield Under Conditions of Medium Soil Salinity

Quantity and quality of yield	Winter wheat		Sugar beet	
	control	4% NaCl	control	4% NaCl
Height of stem in cm	54.3	60.1	—	—
Production of clumps	2.9	3.2	—	—
Length of spike	7.0	8.1	—	—
Number of spikelets in spike	13.3	14.7	—	—
No. of grains in a spike	25.1	31.3	—	—
Wt. of 1000 grains	32.3	33.4	—	—
Yield of grain in cent./ha	20.6	24.6	—	—
Yield of grain in cent./ha	—	—	368.0	413.0
Yield of grain in cent./ha	—	—	175.0	235.0



The effect of soil salinity on the rate of photosynthesis in Sudan grass in mg dry weight per dm^2 leaf surface per hour (average of six measurements), 1) high salinity; 2) medium salinity.

We should also note that under the conditions of our experiment the plants from highly brackish soil were characterized by more abrupt variations in the rate of photosynthesis during the day (diagram). The rate of photosynthesis was higher in these plants during the morning and late afternoon hours than it was in plants from a medium brackish soil. In the middle of the day (from 11 until 2) the reverse was observed: the accumulation of dry weight in plants form a highly brackish soil occurred at a slower rate than it did in plants from a moderately brackish soil. An increase in chlorophyll content was observed in all the plants as the degree of soil salinity increased.

Thus, under the conditions of our experiments in which the degree of soil salinity was increased, the glycophytes exhibited an increase in catalase activity, an increase in chlorophyll content, and a change in the rate of respiration; the direction of the change in the rate of respiration depended on the specific biological nature of the plant as well as the type of salts present in the soil. In one of the plants studied (Sudan grass) an increase in the ratio of photosynthesis was also observed.

Another aspect of our investigation was to study the glycophytes from the viewpoint of increasing their salt-hardiness.

At the present time many methods are known for increasing salt-hardiness in plants by agricultural

engineering and improvement practices: these can play an important role in increasing crop yields on brackish soils. In our investigations we used Genkel's method [22], which, in substance, consisted of a presowing treatment of the seeds with salt solutions. Such a treatment brings about a change in the colloidal-chemical-properties of protoplasm in the embryo, and makes it possible for the plants to endure soil brackishness better during their later stages of growth. Sugar beet and winter wheat Liutestsens 230 were used in the experiments. Swollen seeds of each of these crop plants were soaked for an hour in a 4% sodium chloride solution and then rinsed for half an hour with distilled water.

Our experiments, done under field conditions, indicated the possibility of using this method with the object of increasing the salt-hardiness of sugar beet and winter wheat under irrigation. As one can see from Table 5, under conditions of medium salinity there was an increase in yield of all the experimental plants as compared with the controls. This increase in yield was due to the lesser inhibition of growth processes, and to an increase in the production of clumps, length of the spike, number of spikelets in the spike, number of grains in a spike, and the absolute weight of the grain.

The increase in yield of plants in brackish soils is undoubtedly correlated with an increase in salt-hardiness. This has been demonstrated by direct experiments using microscopic observations and tests of salt-hardiness. Plants from seeds which had been soaked in a sodium chloride solution before planting exhibited an increase in the resistance of the protoplasm of the epidermal cells to the osmotic and toxic effects of salt.

SUMMARY

1. The change in respiration rate of glycophytes as the result of soil brackishness occurred in different directions. The nature of the brackishness was determined. At various medium concentrations, the presence of the SO_4 ion induced an increase in the respiration rate. The chloride ion, regardless of its concentration, always induced a decrease in the respiration rate of the plants.

2. The change in activity of oxidizing enzymes in the glycophytes – polyphenoloxidase and peroxidase – as the result of soil brackishness did not always coincide with a change in respiration rate as the result of the effect of this factor.

3. An increase in catalase activity and chlorophyll content occurred as the soil brackishness increased. An increase in the rate of photosynthesis was also observed in Sudan grass.

4. A presowing treatment of sugar beet and winter wheat seed with a 4% solution of sodium chloride increased the plant yield under irrigation conditions. The increase in yield was related to the increase in the degree of salt-hardiness of the plants.

I wish to express my sincere appreciation to Prof. P. A. Genkel' for his supervision of this work.

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DEGENERATION OF POTATO AND METHODS OF ITS DIAGNOSIS

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Considerable damage to the country's agriculture is caused by the process of degeneration in potato: the potato industry loses up to 10% of its yield because of the distribution of diseases of degeneration [1]. Throughout almost 200 years, investigations have been carried out in an attempt to find causes for the degeneration of potato and to find methods of combating it. At the present time there are two main points of view on the origin of degenerative diseases: ecological and virus. Those who take the ecological point of view [2-4] consider that the process of potato degeneration is conditioned entirely by unfavorable external conditions which cause physiological breakdown and disruption of the metabolism in the potato plant. The defenders of the virus theory [5, 6] consider all degenerative deseases to be due to viruses transferred from one plant to another by the insects living on them (aphids, cicadas).

Without sharply opposing either viewpoint, S. M. Prokoshev considered that the so-called "degenerative diseases of potato and its "virus diseases" are closely interrelated. It is quite possible that the potato, which was brought into cultivation several centuries ago and has been reproduced vegetatively year after year by means of tubers, has become entirely infected with virus by the present time. However, this infection is shown only during the presence of certain unfavorable external conditions.

The first observation on the physiology of degenerated plants indicates the breakdown of their most important living function [7]. Photosynthesis energy is lowered by 30-40%, respiration energy is increased on the average by 50%; the mechanism of the stomatal apparatus is broken down, and in connection with this, the water-sustaining capacity of the plants falls; the chlorophyll content in various types of potato degeneration is lowered by from 22% to 58%; the growth of the plant is retarded.

The breakdown of the most important physiological process is undoubtedly connected with corresponding changes in the metabolism of the plant, which could have been used for a more specific diagnosis of the state of the tubers, since the existing methods of determination of the quality of the seed potato by a superficial examination give a great deviation from the real situation.

The attempt to use the breakdown of carbohydrate metabolism for diagnostic purposes did not give positive results, since the carbohydrates themselves undergo considerable changes during the period of potato storage [8, 9].

The study of the breakdown of the oxidation – reduction system of the degenerated potato has established that there is an increase of oxidase activity [7, 10-13]. On the basis of these data, the method of the "copper test" for diagnosis of the tuber state was proposed [4]; however, the thorough testing of this method [15, 16] showed it to be nonreliable. Also, a method based on the electrometric determination of the potential of potato sap did not receive wide acceptance [17].

The study of nitrogen metabolism gave relatively contradictory results [8, 9, 18-20]. However, in a series of works in this direction the authors' attention was drawn to the work of Friedrich [21]. The German scientist used an unknown biuret reaction for the study of the nitrogen content of diseased and healthy tubers. Here it was found that, when the sap of diseased tubers was mixed with a solution of 1 N KOH and 0.5% $CuSO_4$ and allowed

* Deceased.

TABLE 1

The Nature of the Friedrich Reaction With Potato Tubers

Variety	Color		Out of 100 tubers of the yield of each variety	
	solution	precipitate	1953	1954
Lorkh	Yellow	Yellow	10	20
	Violet	Violet	2	10
	Violet	Yellow	88	70
Berlikhingen	Yellow	Yellow	28	22
	Violet	Violet	1	1
	Violet	Violet	71	73

to stand for a long time, no color change characteristic of the biuret reaction occurred, the color of the solution, as well as of the precipitate formed, remaining violet, while the sap of healthy tubers changed the initial violet coloration of the same reagent to a light yellow color. Often, the author observed even the occurrence of an intermediate reaction—the formation of an abundant yellow precipitate, while the solution remained violet. Such a reaction, according to Friedrich, corresponds in the main to a healthy potato.

It seemed that the author had found a simple and convenient method of diagnosis of the state of potato tubers. However, in the following years this work was not checked experimentally by anyone, and did not receive further development and practical use.

The authors of the present paper undertook to check the data of Friedrich, and in some degree to study the nature of the reaction proposed by him.

First of all, the reaction of Friedrich was checked on 400 separate tubers belonging to two varieties of potato and to two consecutive years of reproduction. During this experiment, the tubers were collected from plants which did not show any evident symptoms of degeneration; thus, in the main, healthy tubers were analyzed.

The reaction itself was carried out in the following way: 2 ml of potato sap, 2 ml of 1 N KOH, and 4 ml of 0.5 % $CuSO_4$ were placed in a test tube. The solution was thoroughly mixed and allowed to stand at room temperature for 20 hours, after which the color of the solution and the color of the precipitate on the bottom were recorded.

As could have been expected, out of 400 tubers studied (Table 1), 386 behaved as healthy (gave a yellow precipitate and either a yellow or a violet solution) and only 14 tubers preserved a violet solution and a violet precipitate during the Friedrich reaction, thus showing themselves as diseased.

TABLE 2

Friedrich's Reaction With Various Methods of Storage of Potato Tubers Variety Berlikhingen

Character of the reaction	Per 100 tubers in storage	
	usual	"warm"
Yellow solution and yellow precipitate	98	63
Violet solution and violet precipitate	0	8
Violet solution and yellow precipitate	2	27

One of the reasons for degeneration of potato is considered to be an incorrect method of storage of the planting material [22], when the necessary low storage temperature (2-3°) was not maintained. It is interesting to check whether "warm" storage of potato would be reflected in the nature of Friedrich's reaction (Table 2).

Of 200 tubers of variety Berlikhingen, 100 tubers were exposed to the action of increased temperatures for 3 days (about 30°), and the second 100 tubers were kept under the usual conditions, i.e., they served as controls. The data of Table 2 show that, after the "warm" treatment, 8 tubers appeared which behaved as

TABLE 3

Friedrich's Reaction on Known Healthy and Degenerated Potatoes

Potato quality	Number of tubers tested	Character of the reaction					
		yellow		violet		intermediate	
		number of tubers	%	number of tubers	%	number of tubers	%
Variety Lorkh							
Healthy	20	20	100	0	0	0	0
Gothic	15	0	0	12	80	3	20
Twisted leaves	15	0	0	15	100	0	0
Striped mosaic	15	0	0	15	100	0	0
Wrinkled mosaic	10	0	0	10	100	0	0
Variety Sovetskii							
Healthy	20	20	100	0	0	0	0
Striped mosaic	15	0	0	15	100	0	0
Dwarfness	10	0	0	9	90	1	10
Twisted leaves	10	0	0	10	100	0	0

diseased, while with the usual storage, diseased tubers were not found at all. The number of tubers with the intermediate character of reaction (violet solution and yellow precipitate) was considerably increased. These tubers may evidently be considered to be changed to some degree as compared with altogether healthy ones. Thus, the conditions of the "warm" storage of tubers affects the character of the Friedrich reaction through the medium of biochemical breakdown in tubers. It is possible that a longer "warm" storage would increase the number of diseased tubers.

For a further check of the possibility of using the Friedrich reaction for diagnostic purposes, we used known diseased and healthy potatoes obtained from the Department of Phytopathology of the Institute of Potato Industry. The results of this check are given in Table 3.

It can be seen from the data of Table 3 that all healthy potato tubers gave a characteristic yellow color with the Friedrich reaction, while, out of 90 degenerated tubers, infected with various diseases (Gothic, twisted leaves, dwarfness, etc) 86 tubers (95%) retained the violet color of solution and precipitate, and only 4 tubers (5%) showed an intermediate reaction character (violet solution and yellow precipitate).

Thus, the character of the Friedrich reaction actually depends on the state of tuber degeneration, and the reaction itself represents a simple and sufficiently reliable method of diagnosis of the state of potato tubers.

Our determination, with the aid of commonly accepted methods [23], of the content of various forms of nitrogen in healthy and in diseased potato tubers (Table 4), as well as in tubers with a different character of the Friedrich reaction (Table 5), did not show any regular quantitative changes. Evidently, the different character of the Friedrich reaction does not depend on the state of nitrogen metabolism in the tubers.

In contrast with the data existing in the literature [24], no regular differences were found in the amino acid content of healthy and degenerated tubers, studied by means of paper chromotography. The other authors also come to this conclusion [25].

We have studied, in the most general way, the factors conditioning the character of the Friedrich reaction. First of all, it was established that the reaction itself is also obtained with other copper salts: instead of 0.5% solution of $CuSO_4$, a 0.5% solution of $Cu(NO_3)_2$, $CuH_2(C_2H_3O_2)_2$ and $CuCl_2$ were used. The salts of other heavy metals did not give the characteristic reaction.

Further, it was shown that the state of the protein in potato sap does not affect the appearance of violet color with the Friedrich reagents: protein, coagulated by boiling, mixed with a corresponding quantity of water, and also partly purified of contaminating substances by means of protein dialysis, gave a deeply violet color independent of the state of the tubers (diseased or healthy). The sap of diseased and of healthy potatoes behaved differently after removal of protein (coagulation and filtration or dialysis). In the case of the healthy potato,

TABLE 4

Content of Various Forms of Nitrogen in Diseased and Healthy Potato Tubers (in % of wet weight)

Potato quality	Nitrogen				Ratios	
	total	protein	non-protein	amino	protein to nonprotein in N	protein to total N
Variety Lorkh						
Healthy	0.404	0.128	0.276	0.21	0.46	0.31
Gothic	0.535	0.242	0.290	0.29	0.83	0.45
Twisted leaves	0.481	0.187	0.294	0.20	0.69	0.39
Striped mosaic	0.460	0.184	0.276	0.12	0.66	0.40
Wrinkled mosaic	0.420	0 133	0.287	0.17	0.46	0.31
Variety Sovetskii						
Healthy	0.546	0.403	0.243	0.40	1.24	0.53
Striped mosaic	0.586	0.294	0.291	0.39	0.99	0.50
Twisted leaves	0.481	0.262	0.219	0.39	1.18	0.54
Dwarfness	0.550	0.268	0.282	0.26	0.87	0.48

the Friedrich reagents gave a bright yellow color and a yellow precipitate was formed; diseased potatoes did not give a precipitate, the sap remained transparent, and became dark green in color.

Thus, it was shown that the yellow precipitate during the Friedrich reaction was not connected with the presence of protein in potato sap, but depended upon the presence of some other organic substance (the potato sap does not give the precipitate after being evaporated to dryness and ashed), which is distinguished by rather low molecular weight (passing through dialyzing membrane).

An acid medium prevents precipitation of the yellow solid, although no essential difference in the pH of the sap of healthy and diseased tubers was found. The sap of healthy tubers does not give such a precipitate in the presence of a strong oxidizer (perhydrol); however, a day later, when the perhydrol was already decomposed (checked by titration with permanganate) an abundant yellow precipitate is again formed. When an energetic reducer (ascorbic acid) is added to the sap with the oxidizer, the retarding action of the latter is nullified. It is interesting to note that a similar yellow precipitate (evidently cuprous hydroxide—CuOH) is formed if the same

TABLE 5

Content of Various Forms of Nitrogen in Potato Tubers with Various Friedrich Reaction Characters (% of dry weight)

Color of solution during Friedrich reaction	Nitrogen				Ratios	
	total	protein	non-protein	aminc	protein to nonprotein in N	protein to total N
Variety Lorkh						
Yellow	1.06	0.52	0.53	0.28	0.98	0.49
Intermediate	1.22	0.57	0.74	0.47	0.89	0.47
Violet	1.09	0.57	0.52	0.45	1.09	0.46
Variety Berlikhingen						
Yellow	1.65	0.74	0.91	0.61	0.81	0.45
Intermediate	1.76	1.10	0.66	0.35	1 66	0.62
Violet	2.01	0.95	1.05	0.57	0.90	0.47

reagents—1 N KOH and 0.5% $CuSO_4$—are added to a solution of ascorbic acid. Dehydroascorbic acid does not give such a precipitate. Also, such reducing substances as glutathione, cysteine, pyrocatechol do not give it.

The impression is formed that there exists in the sap of the healthy potato tuber some organic substance with properties of a reducing factor, which causes the precipitation of the yellow solid, masking the violet color of the biuret reaction. In diseased tubers, infected with such degenerative diseases as Gothic, twisted leaves, striped or wrinkled mosaic etc., this unknown substance is absent, the yellow precipitate is not formed, and the violet color of the biuret reaction appears to a full degree.

We undertook certain studies of the yellow precipitate itself, obtained from the sap of healthy tubers after the preliminary removal of protein (heat coagulation and filtering). It turned out that approximately 30% of it is made up of organic substance, and that, of the 70% inorganic portion, 46.5% is due to copper (determined according to the method of Ermakov [26]). Tests for the presence of nitrogen in the yellow precipitate showed that its content varies around 1.5%.

Further investigations were directed toward determination of the nitrogen-containing organic substances which enter into the consitution of the yellow precipitate. With the aid of a qualitative reaction [27], we established the absence of purine and the presence of amino acids in the yellow precipitate. Evidently the amino acids are not chemically bound, but are merely adsorbed on the yellow precipitate, since a weakly acid-neutralized solution of the latter without hydrolysis, gives a characteristic blue coloration with 1% ninhydrin.

With the aid of paper chromatography, we have become convinced that in almost all cases the yellow precipitate obtained from different parts of the healthy potato contained the following amino acids: aspartic acid, arginine, alanine, valine, histidine, tyrosine. It is possible that this constancy of amino acid content in the yellow precipitate is connected with the common quality of the physicochemical properties, which makes them susceptible to the same degree to the adsorption forces, developing under the conditions of the reaction.

SUMMARY

1. The Friedrich reaction is a convenient and sufficiently reliable method of diagnosis of the state of tubers, with the aid of which it is possible to separate healthy potato tubers from those diseased with one or another kind of degeneration.

2. Formation of a yellow precipitate during the Friedrich reaction with the sap of healthy tubers is connected with the presence in the sap of organic substances with reducing properties. Further investigation of the nature of the substance is required.

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BRIEF COMMUNICATIONS

PRODUCTIVITY OF PHOTOSYNTHESIS IN CROPS SOWED AFTER HARVEST

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Climatic conditions in the western regions of the Ukraine favor the development of crops sowed after the harvest. The length of the growing season in the plants sowed after the harvest consists of 100-110 days without frost, with a heat sum up to 1600° and with a large amount of precipitation, which permits one to obtain a second harvest of a number of crops on the area cleared after harvesting of cereals.

As compared with crops shown during the spring period, plants sowed after harvest extend into the specific condition of constantly lowering temperatures and shortening days. These two factors—temperature and light—play an exceptional part in the life of plants and induce fundamental changes in metabolism.

In order to obtain maximum yield from crops sowed after harvest, it was necessary to determine their metabolic characteristics, first of all, the characteristics of their photosynthetic activity. The experiment was carried out in 1955 under field conditions in the Vinnikovskii area of the L'vov Region.

Sugar and forage beet, forage cabbage, turnips, peas and corn were used as experimental plants. The first three crops were planted as seeds in the fields after harvest of winter rye. Seeds and seedlings were planted on August 1.

Soil moisture at the level of 0-10 cm was 23% at the time of planting, the average air temperature for the first ten days of August was 17°, precipitation was 57.1 mm. The favorable conditions of warmth and moisture favored rapid rooting of seedlings and seed germination. New young rootlets appeared on the roots of cabbage and beets on the third day after planting, and the plant retained almost all its seedling leaves after replanting. Sprouts of pea and corn appeared on August 7. At the same time, new leaves began to develop on the planted seedlings of beet and cabbage, that is, the seedlings began to grow. From this time on, we carried out systematic observations on the development of leaf surface in experimental plants, since the rates of leaf development would, in many ways, determine the yield.

TABLE 1

Leaf Surface Area per Plant in cm^2

Crop	8/15	9/1	10/1	11/1	11/20
Sugar beet	283	1513	3221	5310	4800
Forage beet	132	793	2640	5280	4910
Forage cabbage	168	604	1868	2532	2864
Pea	33	126	963	1450	1910
Maize	36	205	1214	1285	—

The increase of leaf surface in the same plants was measured by tracing the leaf outlines on the paper and subsequently determining the area by weighing. The data obtained are given in Table 1.

To follow the relationship of leaf surface increase to temperature, we give data on the average air

temperature over 10-day intervals during the vegetative period of the plants:

August	September	October	November
17.0	17.6	11.4	1.5
17.2	13.8	8.6	1.8
17.6	13.8	8.8	

As can be seen from the data given in Table 1, increase in leaf surface took place at a rapid rate, particularly in plants which were planted as seedlings. When compared with spring plantings, in the forage sugar beet the death of lower leaves occurred to a lesser degree during the vegetative period, while the appearance of new leaves was observed until the beginning of November, i.e., until the temperature dropped to 1-2°; thus an increase in leaf surface took place up until that time. Throughout all this period, until November, the aerial part of the beet was healthy and was bright green. In the pea and forage cabbage, a small increase of leaf surface through the appearance of new leaves and increase in size of old leaves was noted even in November. The maize at first developed very well, but with the temperature drop below 10° in the beginning of October, the color of the leaves became paler, their ends began to dry up, and vegetative growth ceased almost completely.

The data given in Table 2 of determination of chlorophyll content in leaves of experimental plants show that it was most constant in peas and forage cabbage.

TABLE 2

Chlorophyll Accumulation in Plants Sowed After Harvest (in g/kg wet weight of leaves)

Crops	9/1	10/1	11/1	11/20
Sugar beet	1.6	1.8	1.75	1.4
Forage beet	1 8	1.9	1.8	1.3
Forage cabbage	1.5	1.6	1.6	1.4
Peas	2.0	2.1	2.1	2.0
Maize	1.8	1.1	—	—

TABLE 3

Photosynthetic Productivity (in g per dm^2)

Crops	September	October	November
Sugar beet	10.0	8.0	0.1
Forage beet	14.0	10.0	0.1
Forage cabbage	9.4	12.0	8.5
Peas	9.0	11.0	6.0
Maize	12.0	—	—

Along with the observation of leaf surface development, we also carried out systematic investigations on yield increase, which allowed us to establish the photosynthetic productivity, i.e., the accumulation of yield per unit of leaf surface during various periods of vegetative growth, which differed in their temperature regime. The data obtained are given in Table 3.

The observations show that the crops studied reacted differently to the lowering of temperatures. At the average daily temperature of 8° in October, the photosynthetic yield was sufficiently high in all plants. The yield accumulation in peas and forage cabbage continued in November, too, after the temperature dropped below 5°.

Thus, the data we obtained of the development of leaf surface, chlorophyll content and photosynthetic yield show that when temperature drops below 5° in November, active physiological processes were observed only in peas and forage cabbage, which indicates the greater frost resistance of these plants.

In addition to the quantitative indication of the photosynthetic activity of leaves, the qualitative aspect of this process should also be interesting. Wide application of the isotope-labelling method made it possible to establish that the path of CO_2 assimilation may vary considerably, both according to the nature of the plants and according to the external conditions.

Recent papers [1-3, and others] have established that photosynthesis should be characterized not only by the rate indicators, but also by the formation of various organic substances differing in their composition. With various nutritional conditions, under various external conditions, not only may there be a change in photosynthetic rate, but also a change in the nature of the substances produced during photosynthetic activity.

The experiments we carried out have shown that, under the conditions of sowed after-harvest sowing (short day, lowered temperature of air and soil), there occurs in the plants the formation of mostly labile, easily

TABLE 4

Yield of Crops Sown After Harvest

Cultures	In centners per hectare			In feed units, centners per hectare		
	root	aerial part	sum	root	aerial part	sum
Sugar beet	270.8	187.6	458.4	71.22	42.77	113.99
Forage beet	270.8	162.7	433.5	37.10	22.56	59.66
Forage cabbage	—	433.3	433.3	—	59.75	59.75
Peas	—	297.6	297.6	—	46.92	46.92
Maize	—	166.6	—	—	15.77	15.77

transported structural substances as well as of substances stimulating growth processes ("growth activators", according to the terminology of Sabinin [4]). The plants grew rapidly and rapidly increased their leaf surface; the formation of new leaves was observed until the very last. The synthesis of less labile forms, with an excess of reserved or specific substances ("passive growth," according to the D. A. Sabinin's terminology) was decreased.

Determination of the concentration of dry substance in the sap expressed from roots, which we carried out beginning from October 1, have shown that during October and November, in spite of the increase in size of the roots, the concentration of their cell sap was not increased, remaining at the level of 10-11% for the sugar beet, and of 4-5% for the forage beet. This circumstance indicates to an increase in growth processes over the processes of formation of reserve substances. After the peas bloomed, beginning in the middle of September, the rapid growth and increase in leaf surface was continued, but the seeds developed only poorly.

The direction of photosynthesis in plants shown after harvest towards the formation of transportable labile structural substances indicates that they must be used mostly for forage purposes rather than for technological purposes. Use of such plantings makes it possible to increase significantly valuable vitamin nutrients in the feed supply for cattle, particularly, at that period when they are at a minimum.

The data on the yield, harvested on November 20, given in Table 4, show that the maximal and closely coordinated yield figures were obtained in three transplanted crops: sugar, forage beet, and forage cabbage, which is explained by the rapid increase of their leaf surface.

The planting of seedlings of beet and cabbage allowed considerable prolongation (up to 30 days) of the vegetative period of the plants. Under our conditions of a relatively low temperature and high humidity, the planting took place without injury, the plants easily adapted, and rapidly resumed their growth.

Recalculation to feed units of the yield obtained gave the highest indicators for the sugar beet, which subsequently turned out to be the most profitable forage culture for after-harvest planting, under the conditions planting as seedlings. The most cold-resistant plants under our conditions—peas, forage beet and cabbage—also seem promising.

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ON TRANSLOCATION OF P^{32} IN MEADOW OATGRASS DURING SENESCENCE OF THE SHOOTS

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Previous investigations have established that the separate shoots of the meadow oatgrass clump are in a physiological relationship with each other, and that the clump, up until a certain moment when it enters the phase of a clone in its development, represents, physiologically speaking, a single unit [1, 2].

Further, using the isotope-labelling method, we have refined the information obtained previously, and established the possibility of transfer of substances not only from the older to the younger generations, but also from the younger to the older generations [3].

We studied the rate of outflow of nutrient substances under artificial depression of a number of the shoots of the meadow grass clump. A similar experiment could be significant for the improvement of some agrotechnical methods used in pasture study, such as, for example, mulching. The work was carried out on the experimental station of the V. R. Vil'iams Fodder Institute.

The meadow oatgrass (Festuca pratensis) was used for the investigation. Previously, the shoots in the clump were determined as belonging to various ranks, and then each rank was marked with thread of a certain color. The depression of a number of shoots in the clump was accomplished by means of placing black test tubes on them, preventing the possibility of photosynthesis.

Evidently, from the moment they are covered with the darkened test tubes the shoot ceases to participate in the process of photosynthesis; at the same time, while the shoot stays alive, its nodal roots actively absorb mineral substances from the soil. The utilization of accumulated substances for growth processes and for the formation of new organs does not occur in such a shoot. Because of this, one may believe that the outflow of plastic substances in such shoots may take place more rapidly than in shoots functioning normally. With this consideration in mind, controlled clumps were prepared for comparison, in which transparent, rather than darkened, test tubes were placed on each shoot.

The radioactive isotope of phosphorus— P^{32}, was given to a single shoot in each clump at definite time intervals after placement of darkened and transparent test tubes: 3, 6, 12, 20 and 35 days. The work was carried out according to the accepted method for using this radioactive isotope. Here we took advantage of consultation with Professor A. V. Sokolov.

P^{32} was given as a Na_2HPO_4 solution with a unit of activity of 7.6 μC /ml. At every interval, P^{32} was given to four experimental clumps (covered by darkened test tubes) and to two control clumps (covered with transparent test tubes). Determinations were made with a type B apparatus, using type AC-1 counting tubes of Geiger - Muller. The experimental results are given in the Table.

Three days after being covered with darkened test tubes, the shoots could not be distinguished externally from the controls. There was as yet not even any yellowing of the leaves. Six days later, the leaves in the shoots under darkened test tubes noticeably yellowed. Twelve days later, not only yellowing of the leaves, but a partial senescence of the lower leaves was observed. Twenty days later, the senescence of almost all leaves was observed,—only a leaf coming out from the sheath of the leaf before last, remained alive. After 35 days,

TABLE

Translocation of P^{32} in Clumps of Meadow Oatgrass in Connection with the Length of Darkening of the Shoots in Days (P^{32} content in 100 counts/min per g dry weight)

Clump parts in which P^{32} was determined	Clump No.	3 days	6 days	12 days	20 days
Clumps Without Darkening (Control)					
Third generation shoot with P^{32}	1	42.72	109.65	134.27	204.87
	2	49.10	47.25	100.16	165.38
All other shoots without P^{32}	1	0.47	1.13	1.35	1.46
	2	0.48	0.53	1.00	1.20
Clumps With Darkened Shoots					
Third generation shoot with P^{32}	1	53.37	85.00	36.18	146.28
	2	—	124.33	13.39	127.80
	3	68.56	73.32	64.91	92.63
	4	66.37	54.45	22.81	162.63
All other shoots without P^{32}	1	2.09	2.56	0.76	0.24
	2	—	4.90	0.26	0.59
	3	1.81	2.21	0.97	0.34
	4	3.87	2.39	0.45	0.23

Note: Determination of radioactive phosphorus translocation was terminated after 20-day darkening of the shoots, since after 35-day darkening, all shoots died out.

all shoots inside the darkened test tubes were practically senescent. The shoots inside the transparent test tubes had a normal appearance, even after the longest time interval.

The data given in the table show that translocation of P^{32} from the shoots which began to die into the other plant parts takes place more rapidly than from the normally growing shoots. This translocation is most rapid in the first 5 to 6 days after photosynthesis is stopped in the shoot. Later this process is significantly decreased and ceases, which is connected with the at first partial and then complete senescence of the aerial part of the darkened shoots.

It has also been shown that translation of P^{32} from the younger generation of shoots towards other shoots in the slump of meadow oatgrass is gradually decreased in the normally growing plants (control clumps) as the winter period approaches, which is related to the weakening of the general activity of the plants.

The present investigations were carried out in connection with a study of the effect of mulching on the seed production of meadow oatgrass. The experiments on strip mulching have shown that this method is accomied by an increase in the seed yield of meadow oatgrass and brome grass up to 56% and 50%, respectively; the number of seed-bearing shoots per unit area is increased, the size of the inflorescence is increased, and the quality of the seed is improved (weight per 100 seeds).

It is known that the mulch has a favorable effect on the soil regimes (temperature, nutrient and moisture regimes, biological activity, etc). Our investigations have shown that the positive effect of the mulch is shown not only in this way, but also in the fact that the senescence of the part of the shoots under the mulch favors the improvement of the supply of mineral substances to the remaining shoots in the clump of meadow oatgrass, both because of the increase of the area of nutrition as well as through re-utilization of plastic substances from senescent shoots.

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WATER RELATIONS OF SOME CULTIVATED FORAGE CROPS GROWN ON SALINIZED SOILS

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Salinization of soil has a great and complex effect on plants and their water relations. The effect of salts on the water relation of plants is brought about in several ways. As salts affect the hygroscopic properties and capillarity of the soil and lability of its water, they have a strong effect on the water relations of plants. The high osmotic pressure of the soil solution limits the availibility of water for plants. The penetration of salt ions into plant cells, in turn, affects greatly the water relations of plants. The effect of salts on the water relations of cultivated plants (glycophytes) and of certain native plants (halophytes) is not the same. The cultivated plants differ from the native plants in their relation to soil salinity by the fact that they are less resistant to salinity, since in their evolutionary past they have not adapted to soil salinity. Under the effect of salinization, the osmotic pressure of the cell sap in cultivated plants is increased [1, 2], the osmotic pressure of the plant sap is increased [3, 4]. The transpiration rate is decreased [5-8], and the water content in leaves is decreased [9]. According to several other papers [7, 8, 10], the water content in leaves is increased under the action of salinization.

TABLE 1

Salt Content in Medium Salinized and Highly Salinized Soils of Experimental Plots (in percent of absolutely dry weight of soil)

Degree of soil salinization	Genetic horizons	A plot of perennial forage crops			A plot of Sudan grass		
		dense residue	Cl'	SO_4''	dense residue	Cl'	SO_4''
Medium	A	0.266	0.047	0.012	0.450	0.040	0.012
	B_1	0.729	0.137	0.114	0.375	0.038	0.012
	B_2	0.794	0.144	0.281	1.710	0.166	0.265
	C	1.830	0.192	0.251	1.875	0.178	0.337
High	A	1.253	0.394	0.128	2.875	0.212	0.252
	B_1	1.201	0.347	0.154	2.625	0.148	0.287
	B_2	1.463	0.287	0.332	2.125	0.176	0.312
	C	1.047	0.139	0.025	1.437	0.110	0.237

In these experiments, our problem was to clarify the ways in which water relations are established in various cultivated plants on salinized soil, and to find out which horizons of these soils have a basic significance in the water supply of plants. We were particularly interested in the latter question, since different layers of soil have different amounts of nutrient substances, and play a different role in the nutrient supply of plants. As is shown, the upper layers are the most advantageous in this relation.

Our work was carried out on the P. A. Kostychev Valuiskaia Experimental Improvement Station (Stalingrad Region) in 1953 and 1954. The data given in the Tables refer to the investigations of 1953. We studied cultivated crops: alfalfa, variety Valuiskaia (Medicago sativa), crested wheat grass (Agropyrum cristatum), fescue (Festuca pratensis), couch grass (Agropyrum tenerum) and Sudan grass (Sorghum sudanense). With the exception

TABLE 2

Transpiration Rate of Crops on Medium and Highly Salinized Soils at 11-12 A.M. (in grams per g wet weight per hour)

Conditions of water supply	Degree of soil salinization	Alfalfa	Crested wheat grass	Fescue	Couch grass
	June 5			June 6	
Before watering	Medium	0.384	1.734	1.800	1.464
	High	0.360	1.253	1.590	0.834
	June 17			June 18	
After watering	Medium	2.517	4.040	2.486	1.918
	High	1.179	2.762	1.794	1.220

Note: The transpiration rate of Sudan grass under non-irrigated conditions, measured on July 5, was 1.866 g/ g wet weight per hour on medium salinized soil, and 1.291 g/ wet weight per hour on the highly salinized soil.

of Sudan grass, the plants mentioned were studied under irrigated conditions. During irrigation, they gave high yields of hay; for example, alfalfa gave 230 centners/ hectare. During the first year of our work, the perennial crops (alfalfa, crested wheat-grass, fescue, and couch grass) had been in the second year of their growth, and during the second year of our work, — in the third year of their growth. They grew together as a four-component forage mixture.

The soils in which the crops were studied were light-chestnut, salinized for the second time. The secondary salinization of soils was brought about by the raising of soil water, rich in water soluble salts. The depth of soil waters on the average did not exceed 2 meters. Their salt content reached 25 g per liter of water.

The plants were studied on medium salinized soils and on the highly salinized sections, with areas up to 0.5 hectare, occurring within the the medium salinized region. The medium and highly salinized soils differ very little from each other in morphological structure. The basic difference between them was in the salt content. The chemical analyses carried out have shown that there were considerably less salts in the medium salinized soils than in the highly salinized soils; this difference was particularly great in the upper horizons of the soils. Chlorides were predominant in the upper soil horizons, while sulfates were predominant in the lower horizons (Table 1).

It should be noted that on the highly salinized soil the plants were in a depressed state.

TABLE 3

Water Content in the Leaves on the Medium and Highly Salinized Soils (in percent of wet weight)

Sampling time	Degree of soil salinization	Alfalfa	Crested wheat grass	Fescue	Couch grass
Before watering, June 4	Medium	66.1	55.5	58.2	53.9
	High	62.1	52.7	57.2	52.6
After watering, June 7	Medium	76.0	58.4	66.7	54.8
	High	74.9	57.2	62.7	52.7

Note: Water content in the leaves of Sudan grass under nonirrigated conditions measured on July 5, was 77.7% on the medium salinized soil and 64.6% on the highly salinized soil.

TABLE 4

Daily and Residual Water Deficit in Crops on the Medium and Highly Salinized Soil (in %)

Date of sampling for analysis prior watering	Degree of soil salinization	Alfalfa	Crested wheat grass	Fescue	Couch grass	Sudan grass
		Daily Water Deficit •				
June 4	Medium	41.1	52.0	60.3	41.3	
	High	67.7	61.7	73.1	43.4	
		Residual Water Deficit				
June 5	Medium	23.0	14.5	36.6	4.1	
	High	30.2	17.7	42.8	15.0	
June 15	Medium	15.4	15.7	26.1	15.4	
	High	22.8	17.2	41.4	16.4	

• For Sudan grass under nonirrigated conditions measured on July 5, the value was 9.6% on medium soil, and 17.5% on highly salinized soil.

In studying the water relations of plants, determinations were made of transpiration rate, of the daily and residual water deficits and water content in leaves. Respiration rate was determined by the method of L. A. Litvinov, and water content in leaves by drying them to constant weight at 105°. The daily water deficit was determined at 1 P.M., and the deficit residual at 1 hour before sunrise. Experiments were carried out during dry, sunny weather, mostly in relation to the time of watering: before or after watering.

The results of the experiments have shown that the water exchange of plants takes place in a different way on the highly and medium salinized soil. The transpiration rate was lower and the water content of leaves was lower in plants on highly salinized soil (Tables 2 and 3).

The indicators of water deficit, on the other hand, were higher in all cases in plants on highly salinized soils than in plants on the medium salinized soil (Table 4).

TABLE 5

Heat Resistance of Plant Cells on Medium and Highly Salinized Soils (in °C)

Degree of soil salinization	Alfalfa, June 11	Fescue, June 12	Crested wheat grass June 11	Couch grass, June 12	Sudan grass, July 3
Medium	53.0	53.0	53.5	54.0	57.0
High	51.5	52.0	52.5	53.0	55.0

The lower indicators of transpiration rate and water content in leaves and the higher values of water deficit in plants on highly salinized soil indicate that the water relations are poorer in such plants than in plants grown on medium salinized soil.

The unfavorable effect of the increase of soil salinization on water relations of plants is indicated by the data on determinations of heat stability of cells, which we have carried out according to the method of Genkel' and Tsvetkova [11]. The results of experiments have shown that the heat resistance of plants is lower on highly salinized soils than on medium salinized soils (Table 5).

Kleshnin [12] also observed in his experiments with cotton that the salinization of the substrate lowers the heat resistance of plants. The lowering of heat resistance of cotton observed by Kleshnin evidently took place as a result of the wilting action of salts on plants, since the concentration of substrate salts in his experiment was

considerable (up to 0.5% of chlorine). In those cases in which salts did not produce wilting, the heat resistance of plants may increase [13].

The water relation of the plants which we studied varied greatly in relation to watering, although after watering the great change in moisture occurred only in the uppermost layer of soil, 20 cm thick. The moisture of all lower layers was always high, not less than 80% of the field water capacity, and it was very inconsiderably increased after watering. At the same time, the depth of root penetration into the soil reached, on the average, 70 cm in alfalfa, 65 cm in crested wheat grass, 60 cm in fescue, and more than 70 cm in couch grass.

After watering, as could be seen from the Table given above, the respiration rate of plants was greatly increased, and the water content of the leaves was considerably increased. The daily water deficit was sharply decreased, down to 10%. These facts allow one to consider that the decisive effect on the water supply in perennial grasses is exerted by the uppermost layer of soil. Because of this, to insure the occurance of a normal water regime of perennial plants on salinized soils, and consequently, to obtain a high yield, it is necessary to produce a sufficient moistening of the uppermost layer. Because of this, the watering of perennial forage crops should be carried out in small doses. The forage crops we studied varied greatly from each other in their water relations; the most significant effect of irrigation on water relations was shown by alfalfa and fescue. The crested wheat-grass and couch grass were less reponsive to watering. Sudan grass had a relatively high transpiration rate and small water deficits, even under nonirrigated conditions. These facts indicate a high drought resistance of wheat grass, couch grass and Sudan grass.

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ACCELERATION OF MAIZE DEVELOPMENT BY DIFFERENTIAL PHOTOPERIODIC ACTION WITH A TEN-HOUR LIGHT DAY

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In a paper published previously [1], we noted that medium-maturity varieties of hybrid corn R-3 and B-271, when given a 13-day photoperiodic treatment with a shortened 10-hour day, during the sprouting period, under the conditions of the middle belt of the northern non-chernozem zone, speeded up their development by 6-8 days as compared to the plants on the natural light day, without a noticeable decrease in plant growth and accumulation of organic material. An increase of the photoperiodic treatment by a shortened 10-hour light day from 13 to 28 days had, in plants of the same variety of corn, caused an even greater increase in plant development (from 12 to 13 days), but at the same time brought about a decrease in growth and a decrease in accumulation of organic material by more than one-third as compared to plants left on the natural plant day. The late-maturity varieties of corn, such as Sterling and Krasnodarskaia 1/49, needed a greater number of days of photoperiodic treatment for increase in their development, as compared to the medium-maturity varieties R-3 and B-271. The late-maturity variety Partizanka, while it reacted well to the shortened light day by increasing its development, at the same time insignificantly decreased in growth and in accumulation of the total organic mass.

Other investigators have studied the effect of photoperiodic treatment on development of corn under the conditions of the northern non-chernozem zone [2-11]. But in all papers, as in the one we published earlier [1], the single question has been the optimal number of days of photoperiodic treatment by a shortened light day at which the development of corn plants would be speeded up without noticeable decrease in the yield of ears and of total organic mass.

TABLE 1

Growth and Development of Maize, Variety Partizanka, After Treatment of Young Plants With Different Numbers of Shortened (10-Hour) Light Days (average of 100 plants after harvesting on September 23, 1957)

No. of days of photo-periodic treatment	Plant height in cm	No. of leaves per plant	Wet weight per plant in g			
			stem	leaves	ears	total
Natural light day (control)	183	12	376	169	659	1204
10	158	11	259	118	428	805
15	146	10	264	132	342	738
20	142	10	190	116	285	590
25	147	10	205	112	217	534

To solve this question, in 1956 we took the medium-maturity variety Partizanka as our experimental material, since, according to the studies of the previous year, as shown by data in the literature, this variety was distinguished by a number of positive qualities when cultivated in the middle belt of the northern non-chernozem zone.

The plants were grown in pots of peat compost, which were placed 54 to a box on a layer of earth 4 cm thick, consisting of a mixture of podzolic-turf (50%), compost (35%) and sand (15%). One maize seed was placed in each pot of peat compost. The pots were filled with 1-1.5 cm of earth above the seed. The pots were watered to 60% of the full field capacity of the soil and placed on carts in a glass greenhouse, where the temperature was kept at about 25°C. This moisture level was maintained all the time, up to the time when plants were transplanted into the ground.

The seeds of variety Partizanka, experimental as well as control, were planted in pots on April 30, sprouting began on May 7, and completely-emerged, synchronous sprouts appeared on May 8. From this day, all boxes with plants were divided into 5 groups according to the experimental plan. One group of plants were left all the time on the natural light day (control) and the rest were covered with light-proof boxes at 6 P.M. and were uncovered at 8 A.M. for periods of 10, 15, 20 and 25 days.

After photoperiodic treatment, the plants were transplanted into the open ground on the experimental plot of the Plant Physiology Institute of the USSR Academy of Sciences, Lenin Hills, where they were grown until harvesting. It is necessary to note here that, after transplantation into the ground on May 25, the plants were exposed to unfavorable climatic conditions (a sharp drop in temperature from +25 to +8 and even to +3°, which continued for a week), as a result of which they were somewhat injured (yellowing of leaves) and began to recover only after approximately a week after the weather warmed up.

Throughout the whole vegetative period, physiological observations were carried out on the corn plants; the dates of the beginning of the separate stages of development were noted, which, in their general features, support the data published earlier [1]. Because of this, we will give only the final results of differentiated photoperiodic treatment on accumulation of total organic material (Table 1) and formation of ears (Table 2); we shall also note those peculiarities in growth and development of plants which become clear from the results of these experiments.

TABLE 2

Maturity Characteristics of the Ears of Corn, Variety Partizanka, at the Time of Harvesting (September 23, 1957)

No. of days of photoperiodic treatment	Average No. of ears per 100 plants	Full maturity		Wax maturity		Milk-wax		Milk		Primordial, and those not attaining milk maturity
		No.	in %	No.	in %	No.	in %	No.	in %	
Natural light day (control)	266	19	7.2	87	32.7	128	48.1	32	12.0	126
10	226	87	38.5	94	41.6	38	16.8	7	3.1	102
15	234	153	65.3	55	23.5	26	11.2	—	—	93
20	243	167	68.7	63	25.9	13	5.4	—	—	110
25	216	179	82.2	32	14.8	5	2.4	—	—	58

From the data of Table 1, it can be seen that, as the number of days of photoperiodic treatment is increased, a decrease in plant growth takes place, as well as a decrease in accumulation of organic material. Here it is characteristic of the indicators studied, as compared to the data published earlier [1], that the experimental plants in 1957 reacted more strongly to the photoperiodic treatment both by the acceleration of development as well as by the decrease in their growth.

Thus, for example, in 1956, which was more favorable in its climatic conditions for corn growth as compared to 1957, plant height on the natural light day was 247 cm. while after a 28-day photoperiodic treatment, it was 288 cm; thus growth was decreased by 7.7% by a shortened light day [1].

In 1957, the analagous growth indicators with only a 15-day photoperiodic treatment were, respectively, equal to 183 and 146 cm, or growth was decreased by 20.2%. If, in 1956, the 13-day long treatment by the shortened light day speeded up plant development by 6 to 8 days, without any noticeable decrease in accumulation of organic material [1], in 1957 even a 10-day photoperiodic treatment decreased the wet weight of the

plant by 38.7% (from 1204 to 805 grams). A further increase in the number of days of photoperiodic treatment decreased the accumulation of organic material to an even greater degree (see Table 1).

The data of Table 2 show the degree of acceleration of ear development after various numbers of days of photoperiodic treatment of young corn plants. As can be seen from these data, at harvest time, the ears in the stage of complete and of wax maturity of grain, that is in a state useful as seed, were, in percent: 39.9% on natural light day, 80.1% with 10-day photoperiodic treatment, 88.8% with 15-day photoperiodic treatment, 94.6% with 20-day and 97.6% with 25-day photoperiodic treatment.

Of these, only 7.2% of the ears were in the stage of complete seed maturity when exposed to the natural light day, while with photoperiodic treatment of a 10-hour light day, there were, respectively, 38.6, 65.3, 68.7 and 82.2%.

The average weights of ears (average 100), harvested at the stage of complete and wax maturity of grain, and brought to the proper state for storage (at 10% moisture of grain) are: on natural light day—135.6 grams, with 10-day photoperiodic treatment—131.4 grams, with 15-day—130.9 grams, with 20-day—119.0 grams and with 25-day—126.6 grams.

Thus the photoperiodic reaction of corn plants, variety Partizanka, to a shortened 10-hour light day during the sprouting period has a noticeable effect on acceleration of development and on ear formation (by 10 or 11 days) while the decrease in their weight, up to 15-day-long photoperiodic treatment, is insignificant (by 3-4%).

Regulation of plant development by means of photoperiodic treatment with a shortened (10-hour) day during the period of corn sprouting may find a wide application in selection work, as well as in the production practices of collective forms and Soviet state farms during grain production in the middle belt of the northern non-chernozem zone.

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SETTING PERIOD AND FROST RESISTANCE OF FLOWER BUDS

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The attention of biologists has been attracted to the process of setting of flower buds—the promise of the future harvest. Fruit breeders have empirically developed different methods which change the state of the flower buds in certain critical moments. We have studied the changes which take place in the anatomical structure of buds from the moment of setting. The work was begun in 1935.

If external conditions are unfavorable for the growth of the tree, (drought, leaf injury), cases occur in which flower buds are not set. This is particularly noticeable in pome fruit varieties. For example, in 1937, the formation of flower buds in apple trees began at the normal time, in the end of July, but, because of a severe drought, it was interrupted and was not renewed. There was no flowering of the trees of Renet Simirenko in the state farm "Agromon" in the spring of 1938. The buds looked like flower buds, but only leaves developed from them. An identical phenomenon occurred in 1946 in the orchards of "Krasnyi sadovod" of the Gelendzhikskii Region.

We note three phases in the process of formation of flower buds. The first phase of flower bud formation begins 8 to 10 days after formation of the leaf blade, or 15 to 20 days after the beginning of spring shoot growth. The termination of the first phase takes place at the time when cell division ceases in the growth cone of the bud. In peaches and apricots this coincides with the cessation of the shoot growth; in cherries, plums, apples, and pears, growth ceases 10 to 20 days earlier.

The second phase of flower bud formation begins from the moment of acceleration of cell division in the growing zone and the appearance of a tubercle in the center of the bud. A sharp difference is observed among different varieties. In the flower buds of apple, bird cherries (Cerasus avium), cherries (Prunus cerasus) and plums, the central flower is formed initially. Its development takes place very rapidly. During favorable conditions in the buds, two flowers are rapidly formed almost simultaneously while the rest markedly lag behind in their growth. Up to 30 days passes between the time of termination of the second phase in the first and later flowers in the bud in various years. The number of flowers formed in the flower buds at this time depends on the conditions of tree nutrition and on the functioning of the leaf apparatus and roots. The beginning of the second phase of bud formation takes place about July 20 - August 5 for most of the varieties. The central flower is formed, on the average, in 15 to 20 days. Formation of later flowers occurs in 35 to 40 days in stone fruits (cherry, bird cherry, plum), and in apple it may be spread in some, moister years over 3 months.

Intensity of flower bud formation during the summer period depends upon the moisture context of the soil. At a moisture of 19-22% dry weight the formation which began at the end of July continues up to December and January. In dry years the process of setting ceases and begins anew over periods of various duration from 3-5 up to 15-20 days.

The third phase takes place in the fall-winter period, when the formation of anthers, pistil and stigma is terminated and a swelling of the pistil appears. The formation takes place slowly and gradually, but during warm winters the flowers and the buds are completely formed in December. Usually if November and December were warm, the frosts of the second half of winter cause mass injury of flower buds of all stone fruits. After such winters (1937, 1939, 1940, 1946, 1947, 1949, 1955), the harvests were either very insignificant or non-existent. This is explained by the absence of frost resistance and a high degree of flower formation. If November and December were cold, then the flower formation (third phase) is postponed. During February and March frosts, the flower buds of the stone fruits, except apricots, then retain their frost resistance, are injured in insignificant amounts, and the trees bear fruit. Such were, for example, the years of 1935, 1941, 1948, 1955.

During the third phase of flower bud formation, the effect of the external conditions of the places where the particular variety was developed is particularly clearly expressed. The southern varieties (bird cherry, peach and apricot) were developed under natural conditions of a warm, long autumn which may annually provoke fall flowering. In the process of selection, those forms were selected in which the phases of formation were the longest, so that the danger of the fall flowering was removed so that the buds were kept from being injured. The cherry, apple, and plum were developed in a more northern locality, where, because of the usual fall frost, the second flowering is possible only in special years. Under conditions of the Kuban, they have a short third phase of flower bud formation, which explains the frequent secondary flowering of these varieties. In the peach, there is a greater interval between the second and third phases, and, because of this, there is no secondary fall flowering in peaches under any circumstances. In apricots, the time of initiation of flower buds varies very greatly according to the weather conditions of the year, and the age and variety of the plant. In all fruit varieties, the period of flower bud formation is affected by the age of the tree, its rate of growth, and the type of shoots on which buds are produced. As a rule, the buds are formed in shorter periods on weakly growing shoots and tree.

Frost resistance of flower buds produced on various shoots is not the same. For example, in the winter of 1951-1952, when flower buds of the bird cherry were injured during the March frost, the buds on the spur branches were generally frozen, while on the vegetative shoots, they were retained. In the severe winter of 1953-1954, when injurious frosts took place in December and January, the flower buds of vegetative shoots were frozen, while those on spur branches were retained (see Table).

TABLE

Percent of Living Flower Buds on Various Parts of Bird Cherry in Different Winters

Variety	Spur branches	Vegetative shoots	Spur branches	Vegetative shoots
	1951/52		1953/54	
Aprel'ka	11.0	39.5	72.8	23.2
Denissena zheltaia	5.0	30.0	100.0	80.0
Frantsusskaia chernaia	15.0	73.2	59.0	54.4
Daibera chernaia	16.0	45.5	73.0	7.0

An even sharper picture of the differences in frost resistance may be observed in peaches. In 1948, after a very warm winter, a frost occurred in the end of February. The flower buds on all spur shoots were particularly severely injured, while they were retained on the strongly growing shoots. In 1949, the flower buds on the spur shoots were completely retained, while the buds on the strongly growing shoots were significantly injured. In 1952, and particularly in 1953, the lower buds were retained mostly on vegetative shoots. Such a difference in resistance in flower buds depends on the time of the frost and the state of the buds. The buds formed during the hottest months (July and August) had maximum resistance in the first half of winter. The buds on the stronger growing parts are formed later (in September-October; they have maximum resistance at a time while the buds on short shoots are injured by even insignificant frosts.

In the trees of apricot Krasnoshchekii, the second phase of flower bud formation begins on spur shoots as early as the end of July, and is terminated in the end of August, while in vegetative shoots it begins in August, and is terminated in the beginning of September, and on the secondary shoots (developed through growth of non-wintering buds) the second phase begins at the end of August or beginning of September, and is terminated in October. The injury of flower buds on spur shoots during frosts in the end of the winter of 1947 in Krimskaia Stanitsa was 94%, and 56% of the flower buds perished on the vegetative shoot, while on the secondary shoots, only 31% were killed.

The better the agrotechnique, the more prolonged is the time of formation of fruit buds, since it depends on the rapidity of growth processes. For example, introduction of complete mineral fertilization increased the growth of peach Zolotoi iubilei by 32%. The difference in the time of flower bud formation on the identical types of shoots was 27 days. The fertilized trees varied greatly in buds (initiated at different times), which assured a high yield (fertilized—36.4 kilograms, nonfertilized—21.5 kilograms per tree).

Often a great number of flower buds is initiated on weak, injured, or sick trees, or on trees growing under

conditions of very bad agrotechnique, but they are all formed almost simultaneously, and because of this, hav only a small range of frost resistance, and perish en masse, while on healthy trees, only individual buds perish, which have low frost resistance only at the moment of frost. The large number of flower buds initiated on the shoots of various types over prolonged periods tends to favor a greater frost resistance and the annual fruiting of the trees, a struggle for the preservation of the species.

Sometimes, in certain years, the injury of flowers in buds have different nature. For example, in the winter of 1951-52 there was a sharp frost on March 16 (−13.4°). The formation of flowers and flower buds was terminated at the end of December. The February warmth stimulated opening of the fruit buds. The March frost caused mass injury of flower buds in apricot, cherry, and bird cherry; because of that, there was no harvest in any apricot variety. In cherry and bird cherry the injured parts were primarily the petals, and to smaller degree the stigma and anthers. The injured flowers did not grow, and the flower peduncles were no longer than 0.5 cm, instead of 2-5 cm. Peaches of all varieties showed no more than 20% injury, and gave a yield of more than 100 kg per tree (Krasavitsa−101 kg, Khidistavskii osennii belii−109 kg).

When controlling the growth of the trees, it is possible to regulate the time of initiation of flower buds to a certain degree. In Krasnodarskii kari, July and August are the hottest and driest months throughout the region. Under these conditions, the initiated buds age fast and lose their resistance. One can explain by this the low cold resistance of the stone fruits, which are, in general, predisposed to a deeper bud formation.

Application of agrotechnical methods, improving the growth of fruit trees (watering, fertilization, pruning), prolong the time of initiation of flower buds and create conditions for formation of buds on the fruit tree at various times, so that these buds then have maximum stability at various winter periods.

During frosts in the second half of the winter, the buds initiated on stronger shoots are preserved, while, on the contrary, during early frosts, those that are on the shortened, weakly growing shoots are preserved. It is of this that the struggle for annual stable fruit-bearing of stone fruits in southern regions consists.

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APPLICATION OF COMPLETED INVESTIGATIONS IN THE NATIONAL ECONOMY

MALEIC HYDRAZIDE AS AN INHIBITOR OF ROOT GROWTH IN SUGAR BEET DURING PROLONGED STORAGE

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A considerable part of the harvest of sugar beet roots is placed in prolonged storage each year. This is done primarily to even out the seasonal work of sugar factories, and to establish a rhythm of sugar production, by rationally utilizing the available factory facilities for processing of sugar beet.

However, prolonged storage of sugar beet roots leads to undesirable losses of this valuable raw material. As a result of the metabolism occurring in the roots, the sugar content of the material decreases, and a considerable amount is lost because of the rotting of roots.

Raw material losses become even more significant if growth of roots takes place during storage. To prevent the latter, it was at one time proposed to "trim the roots to a conical shape," by cutting all the eyes off the roots. But such a method of trimming turned out to be very time-consuming, was accompanied by a considerable loss of fresh material, and, because of the large wounded area, made the roots susceptible to the action of decay microorganisms.

Because of these circumstances, the "conical trimming" was replaced by the Iarmoshenko method of root trimming. In this, the leaves were cut off at the base of the petioles, the central (apical) bud was cut out, and all the other eyes located on the root were left untouched. Compared to the "conical trimming," the Iarmoshenko method significantly decreases losses of fresh material, and considerably decreases losses due to rotting of roots. Because it greatly decreases the area of wounding, the Iarmoshenko method turned out to be more successful also because, by cutting only the main bud, and leaving all lateral eyes, it does not greatly disturb the biological integrity of the root, and ensures a higher resistance towards microbes.

In spite of a number of advantages, the Iarmoshenko method still does not solve all storage problems. In many cases, particularly in the southern regions of our country, a beet trimmed by this method soon begins to sprout, which leads to intensification of the living activities of the root, and to a decrease in its sugar content. Treatment of beet with air-slaked lime, practiced in a number of factories, inhibited sprouting, and thus corrected for the deficiency of Iarmoshenko's method, but lime may weaken the protective mechanisms of the roots [1].

In an attempt to find effective means of inhibiting the growth of roots, we tested a number of compounds characterized by high physiological activity: 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, α-naphthalene acetic acid, and the salts of these acids, methyl ester of α-naphthalene acetic acid (preparation M-1), α-naphthyl methyl ester, methyl ester of 2,4-dichlorophenoxyacetic acid, methyl phenylcarbamate, ethyl phenylcarbamate, isopropyl phenylcarbamate, chloroisopropyl phenylcarbamate, cyclic hydrazide of maleic acid, and others. Some of these compounds strongly inhibited the process of sprouting; however, in our experiments the most effective and practically useful compound was found to be the diethanolamino salt of maleic hydrazide (preparation MAH). The most useful method of using MAH turned out to be preharvest spraying of plants (10 to 15 days prior to harvest) with a 0.2-0.3% aqueous solution of this compound, with the addition of a small amount of the accessory substance OP-10, which aids the penetration of MAH into leaves [2-5].

TABLE 1

Average Weight of Roots at the Time of Harvest and Loss of Root Weight After Storage (From October 31 to July 25, 1957)

Experimental treatment	Average wt. per root at the time of harvest, in g	Loss of wt. in % of original	Loss of wt. in % of control
Control	240	14.7	100
MAH, 0.25%	222	10.0	68
MAH, 0.50%	262	8.2	55
MAH, 0.25% + + 0.10% OP-10	237	11.2	76

The first reports on the use of MAH as an inhibitor of growth processes appeared in 1949 [6]. Later, this substance was tested as means of inhibiting sprouting in onion [2, 5, 7, 8], potato [2-5, 9-12], beet [2-5, 13-17] and other plants.

In the present paper, we report the results of our experiments using MAH compound for inhibiting growth of sugar beet roots during prolonged storage. Experiments were carried out in the experimental field of the K. A. Timiriazev Institute of Plant Physiology, USSR Academy of Sciences (Lenin Hills, Moscow) during the period of 1954-1957, inclusively. Because of the correspondence of results in different seasons, we give in the present paper only the data obtained in the season of 1956/57.

MAH was used as the 30% diethanolamino salt. In the experiments we compared the action of 0.25% and 0.5% solutions of the substance, with and without the addition of the adjuvant OP-10, in a concentration of 0.1%. Plants sprayed with a 0.25% aqueous solution of OP-10 were used as controls. The experiments were carried out in triplicate. Fifty plants were used in each series. Treatment was carried out at the rate of 1000 liters of solution per hectare of beet planting. For treatment we used a knapsack-type spraying apparatus with a fine-spray nozzle attachment. Plants were sprayed on October 16.

Throughout the whole subsequent period (up to the moment of harvest) the treated and control plants could not be distinguished from each other. The beets were dug up on October 31, i.e., 15 days after treatment. The roots were separated from the leaves by cutting the latter at the very base of the petioles. The apical and all lateral buds were left untouched. In other words, we treated the beet roots exactly in the same way as when preparing them for planting, in order to obtain seeds. Roots obtained in this way were numbered, weighed, and placed in slatted wooden boxes, and the latter were placed for storage in a partly-underground unheated vegetable storage place. On the day when roots were placed in storage, they were analyzed for sugar content. The roots were kept in this storage place from October 31, 1956 until July 25, 1957. During all this time, the temperature in the storage place was always above zero, and changed within the following limits in the various months: November and December – from 10 to 2°; January, February, and March – from 2 to 6°; April and May – from 6 to 14°; June and July – from 14 to 18°.

TABLE 2

Weight of Sugar Beet Sprouts at the Termination of Storage

Experimental treatment	Weight of sprouts per 50 roots in g	In % of root weight	Weight of sprouts in % of control sprouts
Control	170	1.4	100
MAH, 0.25%	105	0.9	61
MAH, 0.50%	38	0.3	22
MAH, 0.25% + + 0.1% OP-10	90	0.7	52

After termination of the storage period, the size and weight of sprouts was measured, the weight loss of the roots was noted, and the degree of rotting and the sugar content of the roots were determined. Part of the roots from each of the treatments was planted in the field for further observations.

Data on the average weight of roots at the time of harvest and on weight loss during storage are given in Table 1.

It can be seen from Table 1 that treatment of beet leaves considerably decreases weight losses during the storage period (by 24-30% at 0.25% MAH and by 45% at 0.5% MAH concentration). Treatment of sugar beet with MAH solution does not affect the size and weight of roots, if spraying is carried out 15 days prior to harvest (see Table 1). If treatment is done a month or more before harvest, then 0.5% MAH solution may somewhat inhibit root growth: in our experiments, the average weight of roots in that treatment was 86-88% of the control root weight.

TABLE 3

Effect of MAH Content of Sugar in Sugar Beet Roots (initial, and after 9 months of storage)

Experimental treatment	Initial roots			Roots at the end of storage, calculated on initial basis			Retained, in % of initial		Retained, in % of control	
	reducing sugars	sucrose	sum of sugars	reducing sugars	sucrose	sum of sugars	sucrose	sum of sugars	sucrose	sum of sugars
Control	0.75	17.00	17.75	0.88	15.38	16.26	90.4	91.6	100	100
MAH, 0.25%	0.95	16.43	16.38	0.80	15.81	16.61	96.2	95 5	102.7	102.1
MAH, 0.50%	0.68	17.05	17.73	1.01	15.94	16.95	93.4	95.6	103.6	104.2
MAH, 0.25% + + 0.1% OP-10	0.73	16.69	17.42	0.73	16.59	17.32	99.4	99.4	109.9	108.4

As can be seen from Table 2, the preharvest treatment of sugar beet leaves with 0.5% solution of MAH sharply decreased the formation of the sprouts (to less than one-fourth of controls). Spraying with 0.25% MAH solution together with OP-10 turned out to be somewhat more effective than spraying with 0.25% MAH alone (52% instead of 61% in the latter case). It should be noted that the smallest number of fungus-infected roots was found in the treatment of 0.25% MAH + OP-10 (16.7% instead of the 33% in the control).

In Table 3, the sugar content of sugar beet roots at the beginning and end of the storage period is given. At the end of storage, the average sample was taken only from the healthy well-preserved roots.

TABLE 4

Growth and Development of Sugar Beet Planted in the Field After Being Treated with MAH (after 9 months of storage)

Control treatment	Sprouted roots	Average wt. of green mass of plant in g	Average number of seed-bearing shoots per plant
Control	100	651	13
MAH, 0.25%	20	107	None
MAH, 0.50%	0	None	None
MAH, 0.25% + +OP-10	50	572	None
MAH, 0.5% + +OP-10	0	None	None

The data given show that for the 9 months of storage the sucrose content of control roots decreased by 10%; the smallest sucrose loss was found to occur in the treatment with 0.25% MAH with OP-10 added. The sucrose content here was almost 10% greater than that of the control, but the total sugars sum was 8.5% greater. In the treatment with 0.25% MAH without OP-10, the sucrose content and total sum of sugars was somewhat higher than in the roots of the control (correspondingly +2.7% and+ 2.1%), but lower than in the treatment with 0.25% MAH + OP-10.

If total losses after storage are calculated, the sucrose loss per 100 kg of sugar beet would be 2.155 kg for the control, and for the 0.25% MAH + OP-10 treatment it would be 0.116 kg, thus almost one-twentieth of that in control.

After planting in the field, all the control roots gave well-developed seed-producing plants. The roots treated with MAH, however, behaved differently: with 0.25% MAH solution + OP-10, only 50% of the roots began to grow, while in the treatment with 0.5% MAH, only solitary roots appeared (Table 4).

It should be noted that roots, the leaves of which were treated in the fall with 0.25% MAH solution with OP-10 added, and which, after being planted in the field in the following summer, began to grow, formed a great vegetative mass; however, formation of seed-bearing shoots was altogether lacking.

SUMMARY

Preharvest treatment of sugar beet plants with solution of MAH strongly inhibits growth of the roots of this plant during prolonged storage. The roots can be stored without removing the buds and hence without injuring the root head by employing the aforementioned preparation.

In contrast with the roots of untreated plants, those of plants treated with MAH are preserved in a better

state, lose less sugar and rot to a smaller extent. Good results can be obtained by spraying the plants with a 0.25% solution of MAH two weeks before harvest.

The results obtained indicate that large scale experiments are desirable to test MAH preparations as a means of prolonging the storage of root beets.

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METHODS

QUANTITATIVE DETERMINATION OF CATECHINS IN PAPER CHROMATOGRAPHIC SEPARATION

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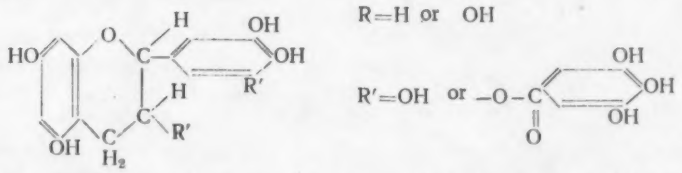
Catechins are the simplest representatives of tannic substances of the condensed series. They are widely distributed in plants, and are found in tea, grape, gambier, willow, pine, larch, and in fruits of apple, pear, peach, etc. Catechins and their oxidized derivatives play an important role in the preparation of black tea [1] and in the processes of wine-making [2]. Epiforms have a high biological activity. They regulate the permeability of blood vessels and increase the elasticity of capillary walls, and for this reason may be included among the substances of vitamin P group [3, 4].

By their chemical nature catechins are derivatives of diphenylpropane or 1-phenylchromone:



The presence of two asymmetric carbon atoms in the catechin molecule, as well as their capacity to form esters with gallic acid (gallates), explains the multiplicity of different types of compounds of this class.

In connection with the constantly developing investigations of the formation and transformation of different polyphenols in plants [5-7], catechins, in particular [8, 9], the question of technique becomes very important. A most promising method is that of paper chromatography, which enables rapid separation of small amounts of the most complex mixtures of natural substances.

A sharp separation of catechins is obtained with the use of acidified one-phase solvents, such as n-butanol-$CH_3COOH-H_2O$ (40 : 10 : 22), 2% CH_3COOH [10], or n-butanol-$CH_3COOH-H_2O$(40 : 12 : 28) [11].

As a result of the unidimensional chromatographic run on paper (solvent: n-butanol-$CH_3COOH-H_2O$), it was possible to separate a complex of catechins from tea plant leaves-consisting of L-epigallocatechin, D,L-gallocatechin, L-epicatechin, D,L-catechin, L-epigallocatechingallate, L-gallocatechingallate, and L-epicatechingallate,-into five separate spots, three of which are individual compounds, and two of which represent a mixture of optical isomers.

During a comparative study, such a separation is usually sufficient, since D,L-catechin and L-gallocatechingallate are not major components of the complex, and are present in relatively small amounts.

The first attempt to use paper chromatography for the quantitative determination of catechins was made by Oshima, Nakabayashi, and Nishida [12]. These authors measured catechin concentration by the area of spots in unidimensional chromatograms. This method is not very precise and the authors themselves called it semi-quantitative. Forsyth [13] determined the catechin content in cocoa beans by cutting out the corresponding spots on chromatograms, and titrating them with 0.01 N $KMnO_4$ solution. The amount needed for titration of control substances was determined in parallel samples. The method of W. Forsyth, beside being inexact, is also

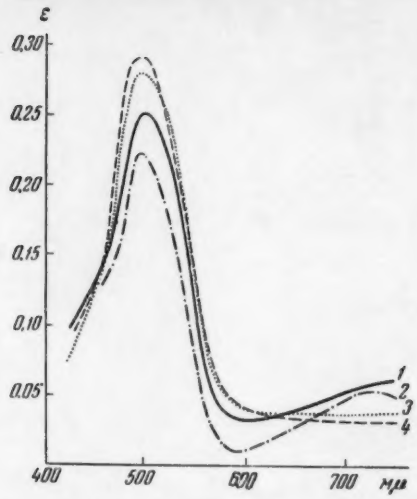


Fig. 1. Absorption spectra of colored complexes of catechins. 1) L-epicatechingallate (41.1 μg); 2) L-epicatechin (23.4 μg); 3) L-epigallocatechingallate (50 μg); 4) L-epigallocatechin (36 μg).

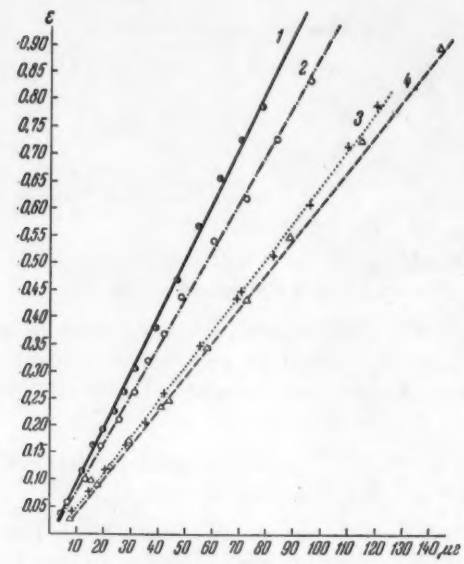


Fig. 2. Calibration curves for catechins (reaction with vanillin). 1) L-epicatechin; 2) L-epigallocatechin; 3) L-epicatechingallate; 4) L-epigallocatechingallate.

nonspecific, since $KMnO_4$ will oxidize other substances which may remain in the zones of individual catechins after chromatographing.

The method developed by Dzhemukhadze and Shal'neva is more precise [14]. In this method, separation is carried out by radial chromatography in a nitrogen atmosphere. The zones of the separate catechins are cut out and eluted with 96% ethanol. The concentration of catechins in the eluents is determined colorimetrically, by reaction with vanillin, using the photoelectric colorimeter of Shipalov's system (blue filter) for this purpose.

In our work, however, when we needed to make quantitative determinations of the catechins by means of paper chromatography, we were unable for a number of reasons, to use the data of K. Dzhemukhadze and G. Shal'neva.

These authors do not give tables for calculation of the content of separate catechins from the experimental indicators, but give only a small-scale drawing of the calibration curves, which cannot be utilized for practical use. In addition, the type of apparatus used by these authors has not been widely used in laboratory techniques.

Moreover, it was found that the absorption peak of catechin complexes stained with vanillin is located, not at 4450-4600 A (blue filter) [14], but at 4900-5000 A (blue-green filter), and the change in the filter increases the sensitivity of the apparatus on the average by 50%. In constructing calibration curves for L-epigallocatechingallate and L-epicatechingallate, we have again found significant differences from the data of Dzhemukhadze and Shal'neva [14].

All this had stimulated us to develop the simple and sufficiently reliable method of quantitative determination for catechins given below, using the simplest descending paper chromatography.

Experimental Method

Paper. Catechin separation was carried out by means of descending chromatography on "Chromatographic No. 3" paper (handmade), an experimental lot from TsNIIB* (Leningrad). For removing heavy metal admixtures, a 20% aqueous solution of formic acid was passed through the paper for 48 hours.**

*Central Scientific Research Institute of the Pulp and Paper Industry.

**This method of washing the paper was recommended by E. Vyskrebentseva. It is simpler and more reliable than the alternative treatment of paper by alcoholic alkali and hydrochloric acid [14].

No. 3 paper has good separation properties and a sufficiently large holding capacity, being better in these two qualities than the "slow chromatographic" and "fast chromatographic" papers.

TABLE 1

Table for Calculation from Photometer Data of the Content of L-Epicatechin and D,L-Catechin in the Sample Taken for Analysis (S = 50 blue-green filter, readings on the red scale, 0.5 cm cuvettes)

Scale reading E	Amount of catechin in μg	Scale reading E	Amount of catechin in μg	Scale reading E	Amount of catechin in μg	Scale reading E	Amount of catechin in μg	Scale reading E	Amount of catechin in μg
0.04	5.0	0.20	21.0	0.36	36.3	0.52	51.5	0.68	66.8
0.05	6.0	0.21	21.9	0.37	37.2	0.52	52.5	0.69	67.8
0.06	7.0	0.22	22.9	0.38	38.1	0.54	53.5	0.70	68.8
0.07	8.0	0.23	23.9	0.39	39.1	0.55	54.4	0.71	69.8
0.08	9.0	0.24	24.9	0.40	40.0	0.56	55.4	0.72	70.7
0.09	10.0	0.25	25.8	0.41	41.0	0.57	56.4	0.73	71.7
0.10	11.0	0.26	26.8	0.42	42.0	0.58	57.3	0.74	72.7
0.11	12.0	0.27	27.7	0.43	43.0	0.59	58.3	0.75	73.6
0.12	13.0	0.28	28.7	0.44	43.9	0.60	59.2	0.76	74.6
0.13	14.0	0.29	29.6	0.45	44.9	0.61	60.2	0.77	75.5
0.14	15.0	0.30	30.6	0.46	45.8	0.62	61.1	0.78	76.5
0.15	16.0	0.31	31.5	0.47	46.8	0.63	62.1	0.79	77.4
0.16	17.0	0.32	32.5	0.48	47.8	0.64	63.0	0.80	78.4
0.17	18.0	0.33	33.4	0.49	48.7	0.65	64.0	0.81	79.3
0.18	19.0	0.34	34.4	0.50	49.7	0.66	64.9	0.82	80.3
0.19	20.0	0.35	35.3	0.51	50.6	0.67	65.9	0.83	81.2

Solvent. As a solvent we used the mixture selected earlier [11], n-butanol – CH_3COOH – H_2O (40:12:28 by volume). This mixture separates on standing for a few days, and because of this we used it only in a freshly prepared state.

Developing Agent. For developing control strips of chromatograms and for the quantitative determination of catechins after their elution from chromatograms, a 1% vanillin solution in concentrated hydrochloric acid (chemically pure) was used. [15]. Such a solution, when kept in a refrigerator, should retain its lemon-yellow color, without changing, for at least 24 hours.

TABLE 2

Table for Calculation from Photometer Readings of the Content of L-Epigallocatechin and D,L-Gallocatechin in the Sample Taken for Analysis (conditions the same as in Table 1)

Scale reading E	Amount of catechin in μg	Scale reading E	Amount in catechin in μg	Scale reading E	Amount in catechin in μg	Scale reading E	Amount in catechin in μg	Scale reading E	Amount of catechin in μg
0.04	5.1	0.20	23.1	0.36	41.2	0.52	59.6	0.68	78.0
0.05	6.1	0.21	24.2	0.37	42.4	0.53	60.8	0.69	79.1
0.06	7.2	0.22	25.3	0.38	43.6	0.54	61.9	0.70	80.2
0.07	8.4	0.23	26.5	0.39	44.8	0.55	63.1	0.71	81.4
0.08	9.6	0.24	27.7	0.40	45.9	0.56	64.3	0.72	82.5
0.09	10.7	0.25	28.9	0.41	47.0	0.57	65.4	0.73	83.6
0.10	11.7	0.26	30.0	0.42	48.1	0.58	66.5	0.74	84.8
0.11	12.9	0.27	31.1	0.43	49.3	0.59	67.6	0.75	85.9
0.12	14.0	0.28	32.2	0.44	50.5	0.60	68.8	0.76	87.0
0.13	15.1	0.29	33.3	0.45	51.7	0.61	70.0	0.77	88.2
0.14	16.2	0.30	34.5	0.46	52.9	0.62	71.2	0.78	89.3
0.15	17.4	0.31	35.6	0.47	54.1	0.63	72.4	0.79	90.4
0.16	18.5	0.32	36.7	0.48	55.2	0.64	73.5	0.80	91.6
0.17	19.7	0.33	37.9	0.49	56.3	0.65	74.6	0.81	92.8
0.18	20.8	0.34	39.0	0.50	57.4	0.66	75.8	0.82	93.9
0.19	22.0	0.35	40.1	0.51	58.5	0.67	76.9	0.83	95.1

TABLE 3

Table for Calculation from Photometer Readings of the Content of L-epicatechingallate in the Sample Taken for Analysis (conditions the same as in Table 1)

Scale reading E	Amount of catechin in μg	Scale reading E	Amount of catechin in μg	Scale reading E	Amount of catechin in μg	Scale reading E	Amount of catechin in μg	Scale reading E	Amount of catechin in μg
0.04	7.2	0.20	31.6	0.36	55.9	0.52	80.0	0.68	104.2
0.05	8.8	0.21	33.1	0.37	57.4	0.53	81.5	0.69	105.7
0.06	10.3	0.22	34.6	0.38	58.9	0.54	83.0	0.70	107.2
0.07	11.8	0.23	36.1	0.39	60.4	0.55	84.5	0.71	108.7
0.08	13.3	0.24	37.6	0.40	61.9	0.56	86.0	0.72	110.3
0.09	14.9	0.25	39.1	0.41	63.4	0.57	87.5	0.73	111.8
0.10	16.4	0.26	40.6	0.42	64.9	0.58	89.0	0.74	113.2
0.11	17.9	0.27	42.2	0.43	66.5	0.59	90.5	0.75	114.7
0.12	19.4	0.28	43.8	0.44	68.0	0.60	92.0	0.76	116.3
0.13	20.9	0.29	45.6	0.45	69.5	0.61	93.5	0.77	117.8
0.14	22.5	0.30	46.8	0.46	71.0	0.62	95.0	0.78	119.4
0.15	24.0	0.31	48.3	0.44	72.5	0.63	96.5	0.79	121.0
0.16	25.5	0.32	49.8	0.48	74.0	0.64	98.0	0.80	122.5
0.17	27.0	0.33	51.3	0.49	75.5	0.65	99.6	0.81	124.0
0.18	28.5	0.34	52.9	0.50	77.0	0.66	101.4	0.82	125.5
0.19	30.0	0.35	54.9	0.51	78.5	0.67	102.8	0.83	127.0

Catechins. Crystalline catechins were obtained by fractionation of tannin from tea leaves on silica gel columns [16]: L-epigallocatechin, melting point 218°; L-epicatechin, m.p. 235°; L-epigallocatechingallate, m.p. 213°; $[d]_D$ 20° = − 185° (water); L-epicatechingallate, m.p. 135°; $[d]_D$ 20° = − 188° (water-ethanol mixture, in the ratio 1 : 1).

Quantitative determination of catechins was done on a graduated Pulfrich photometer. 1.0 ml of a catechin solution was mixed in a test tube with 5 ml of 1% vanillin in concentrated HCl, and the intensity of the red color formed was measured in the photometer, in cuvettes 0.5 cm thick. Absorption spectra of catechin-vanillin complexes are given in Fig. 1.

TABLE 4

Table for Calculation from Photometer Readings of the Content of L-epigallocatechingallate and L-gallocatechingallate in the Sample Taken for Analysis (conditions the same as in Table 1)

Scale reading E	Amount of catechin in μg	Scale reading E	Amount in catechin in μg	Scale reading E	Amount of catechin in μg	Scale reading E	Amount of catechin in μg	Scale reading E	Amount of catechin in μg
0.04	8.3	0.20	34.2	0.36	95.6	0.52	85.2	0.68	110.8
0.05	10.0	0.21	35.8	0.37	61.2	0.53	86.8	0.69	112.4
0.06	11.5	0.22	37.4	0.38	62.8	0.54	88.4	0.70	114.0
0.07	13.1	0.23	39.0	0.39	64.4	0.55	90.1	0.71	115.6
0.08	14.6	0.24	40.6	0.40	66.0	0.56	91.7	0.72	117.2
0.09	16.3	0.25	42.3	0.41	67.6	0.57	93.2	0.73	118.8
0.10	18.0	0.26	43.8	0.42	69.1	0.58	94.9	0.74	120.3
0.11	19.5	0.27	45.3	0.43	70.6	0.59	96.6	0.75	121.9
0.12	21.0	0.28	46.8	0.44	72.3	0.60	98.2	0.76	123.6
0.13	22.6	0.29	48.3	0.45	74.0	0.61	99.8	0.77	125.2
0.14	24.2	0.30	50.0	0.46	75.6	0.62	101.5	0.78	126.8
0.15	25.8	0.31	51.7	0.47	77.2	0.63	103.0	0.79	128.4
0.16	27.4	0.32	53.3	0.48	78.9	0.64	104.6	0.80	130.0
0.17	29.1	0.33	54.9	0.49	80.5	0.65	106.1	0.81	131.6
0.18	30.8	0.34	56.5	0.50	82.0	0.66	107.6	0.82	133.1
0.19	32.5	0.35	58.0	0.51	83.6	0.67	109.2	0.83	134.7

TABLE 5

Check for the Precision of the Method for Separate Catechins

Catechins	μg applied to paper	μg after chromatog. + elution	% found
L-epicatechin	56.0	51.5	92.0
L-epigallocatechin	88.0	78.5	89.2
L-epigallocatechingallate	55.0	48.3	88.0
L-epigallocatechingallate	72.0	64.9	90.1
Mean loss		—	10.2%

It can be seen from Fig. 1 that under these conditions all catechins give a clearly expressed absorption peak at approximately 5000 A (S = 50 blue-green filter, with maximum transmission at 4900 A). At 4600 A (S = 47 blue filter, maximum transmission at 4580 A), absorption is sharply decreased. Taking this data into consideration, we have used the S = 50 filter in all further determinations in construction of calibration curves.

In Fig. 2 are given calibration curves for four of the most typical catechins: L-epigallocatechin, L-epicatechin, L-epigallocatechingallate and L-epicatechingallate, obtained from solutions of the pure catechins. The linear nature of the curves obtained indicates that they obey Beer's law.

The other components of the tea catechins complex may be calculated from the four basic calibration curves. In this case, D,L-epigallocatechin will behave as L-epigallocatechin in the vanillin reaction, D,L-catechin, as L-epicatechin, and L-gallocatechingallate, as L-epigallocatechingallate.

Below are given tables for data calculation which were obtained on the basis of the calibration curves given in Fig. 2.

As can be seen from the tables given (1, 2, 3 and 4), the reaction of catechins with vanillin is very sensitive, and makes it possible to detect as little as 5-10 μg of substance.

In practice, the critical step is the elution of the catechin spots from chromatograms. Because catechins may be easily oxidized, and because of their adsorption on the paper, at this stage, in particular, significant losses of substance may be encountered.

Two elution methods were tried:

1) Steeping of finely cut chromatograph sections in three changes of 96% ethanol at 30-40°, and

2) elution by passing a flow of 96% ethanol, or ethanol with 3% of acetic acid added, along a twisted cellulose thread through the cut-out spot.

The first method gave higher and consistent results. It results in recovery in the eluate of 88% to 92% of the catechins applied to the paper (Table 5). For comparative determinations, such precision must be considered adequate.

As can be seen from Table 5, it is necessary to correct the results obtained for the 10% loss in the process of chromatographing and elution. For obtaining more precise absolute results, the elution should be carried out in an atmosphere of an inert gas [14].

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BOOK REVIEW

FOLIAR NUTRITION OF PLANTS

F. F. Matskov

USSR Academy of Sciences Press, Kiev, 1957

In connection with the wide application of mineral fertilizers in our country, many scientific institutions are developing new methods which lead to increased size and higher quality of yields. One of the promising methods of sectional introduction of fertilizers is the foliar nutrition of plants. Interest in this method has increased to an extraordinary degree, particularly in recent years.

The appearance of the book by F. F. Matskov, "Foliar Nutrition of Plants," is welcomed by readers, as there has been no summary of this field. When one considers the divergence between results of laboratory investigations and field experiments using this method, along with the differing opinions of different investigators, as well as the practical proposals which have been made, the appearance of a review work in this field becomes very timely.

The book presents the results of a systematic investigation by the author and his collaborators beginning in 1933, as well as numerous data from the literature. The material is successfully arranged in four chapters, which illuminate theoretical questions on the foliar nutrition of plants, general questions (the supplementary nutrition of plants cultivated in the field) and finally, the question of foliar nutrition as a means of diagnosing the plant's fertilizer needs.

The author's intention of avoiding empiricism and a blind seeking of the most successful methods of application of foliar nutrition in agricultural practice is particularly valuable. In the theoretical section, the questions of the mechanism by which mineral substances are absorbed by plant leaves, and of paths and velocityof mineral translocation in the plant are thoroughly treated. The results of microchemical analysis in establishing the mechanism of penetration and the speed of translocation of nutrient elements, depending on the composition of the salt, its concentration, and the pH of the medium, are systematically presented. The development of this investigation is reflected in subsequent macrochemical analyses, and, further, in the application of the isotope-labeling method. The principal correspondence of the mechanism of ion absorption by the root system and by plant leaves is established.

In consideration of the part played by leaf age in absorption of the basic nutrient elements, it seems to us that the great energy of absorption of nitrogen, potassium, and phosphorus by old leaves as compared with young ones, is a little too categorically stated (pp. 18, 19 and 24). At the present time, there are sufficient data in the literature in which the isotope-labeling method has shown the opposite, i.e., more energetic absorption and translocation of nutrient elements in the young leaves of various plants. There is no real assurance of the precision of the determination of the amount of P^{32} applied to the leaf surface, by means of counts made after drying of the nutrient solution applied to the leaf surface (page 25), considering the greater speed of the first stage of absorption, and also the probability of translocation of absorbed ions inside the leaves (which necessitates a correction for self-absorption).

Insofar as the role of the water supply of leaves in P^{32} absorption is concerned, it is stated (page 27: leaves given less water absorbed more phosphorus and translocated it in larger amounts to other parts of the plant than did plants supplied with more water. This position agrees poorly with the investigations of other authors on the translocation of substances in the plant and on the part played by the conditions of the water regime. Besides, on page 108, the author points out the inexpediency of carrying out supplementary foliar feeding in periods of very dry weather (connecting the weak effectiveness of supplementary feeding with the absence of night dew).

Interesting material is presented in the part dealing with the effect of foliar nutrition on the physiological processes taking place in plants: photosynthesis, respiration and enzyme activity. The positive effect of the supplementary foliar nutrition on the processes of photosynthesis and respiration is shown. The possibility of regulating, by this means, the direction of action of the enzymatic apparatus of plants is established. It becomes clear that it is possible through foliar nutrition to increase the plant's resistance to unfavorable external conditions (low temperatures, drought, soil salinity).

The author does not sufficiently work out the question of controlling the direction of enzyme action in plant leaves at various stages of the life cycle, as well as of leaves belonging to different whorls at a given stage of development, which is significant for the choice of the fertilizer composition used during foliar nutrition with the purpose of producing a directed change of enzymatic processes. The statement (page 46) that hydrolytic processes begin to predominate in leaves in the second half of the vegetative season is completely correct, but, together with this, it is necessary to take into account the different quality of the leaf series at various stages of plant development, i.e., the presence of old leaves (with a dominating hydrolytic fraction) in the young organism and of young leaves (with a dominating synthetic fraction) in the older plant which has, however, not yet ceased its growth. It seems that the statement about prolonging the youth of the plant and prolonging of its vegetative period by a high level of nitrogenous nutrition, and accelerating ageing and the beginning of senescence by a high level of phosphorus nutrition (page 50) is a little too general. It is known (Chailaikhian, Volodarskii, et al.)that not all plants change the rate of their development in a similar way when the level of nutrition, particularly of nitrogenous nutrition, is changed.

The author of the book gains the complete confidence of the reader by the thorough description of the methods of experimental work, and by the calm and objective description of experiments which gave both positive and negative results. While he mentions the unusually high increase of sugar concentration in beet roots reported in the literature, the author of the book also gives his own, more modest, results (page 74). One cannot but approve the call of F. F. Matskov to the workers of scientific institutions and to practical agriculturalists to publish, in sufficiently full and detailed form, the experiments in which the positive effect expected was not obtained from foliar nutrition.

In the work reviewed great attention is given to the practical side of the application of foliar nutrition, which enables one to judge the possibility of industrial use of this new method, its effectiveness, the degree of possible mechanization and the expectation of profit.

In considering foliar nutrition as a method of diagnosing the plant's fertilizer needs, the author of the book supposes that the response of the plant's growth processes to foliar nutrition is a more reliable indicator of its need of a particular nutrient element than are the results of chemical analysis of its leaves or of any other organs (page 234). This method of diagnosis is very tempting in its simplicity, but it cannot have universal significance, because the processes of growth of the plant respond in different ways to change in nutrient conditions at various stages of its life cycle, and the growth processes also change to a different degree after similar changes of the level of nutritional elements.

In view of the fact that general questions of plant metabolism and of the mineral nutrition of plants are treated in the book, one regrets that the works of D. N. Prianishnikov, A. V. Vladimirov, and I. I. Kolosov are absent from the bibliography.

The conclusion of F. F. Matskov's book is very comprehensive. There, he objectively demonstrates the limitation of our knowledge of a number of questions closely connected with the theoretical basis of foliar nutrition and its practical application.

The paths for further investigations are indicated in the conclusion. One would have wished that this collection of questions had been supplemented by a study of foliar nutrition in close connection with the biology of plant development, of the changing needs in nutrient elements according to the stage of development, and of the critical periods in the mineral nutrition of plants, differentiating them in relation to vegetative and reproductive organs, as well as in relation to the rate of development of the leaf apparatus and to the different quality of the leaves composing it.

As a whole, F. F. Matskov's book is without a doubt a valuable one. It gives the reader a clear conception of the state of this possible method of increasing yields, above all directing the attention of plant physiologists to those aspects whose study is necessary for a more rational application of foliar nutrition under industrial conditions.

L. G. Dobrunov

REPORT

INTER-REPUBLIC CONFERENCE IN TASHKENT ON METHODS FOR STUDY OF THE PHYSIOLOGY AND BIOCHEMISTRY OF THE COTTON PLANT

From December 17 to 19, 1957, a conference was held in Tashkent on the methods of physiological and biochemical investigation of the cotton plant, organized by the Commission on Coordination of Scientific Research in Cotton Industry of the Academy of Sciences, UzSSR.

Physiologists and biochemists of the cotton-growing republics (Uzbek, Tadzhik, Turkmen Kazakh, and Kirgiz SSR), as well as representatives of the K. A. Timiriazev Institute of Plant Physiology AN SSSR and the V. L. Komarov Botanical Institute AN SSSR, took part in the conference.

In a survey report by V. S. Shardakov, the general problems of methods of physiological and biochemical investigations were presented. Considerable attention was given to the advantages of modern methods—their higher sensitivity, the acceleration of the process of analysis, and the possibility of penetrating into fields of the vital activity of the plant organism, which earlier had been inaccessible. Problems of the organization of physiological and biochemical investigation were illuminated in detail, as were the reliability of average samples, methods of recalculation of analytical data, and methods of mathematical treatment of data.

The subsequent reports dealt with problems of methods in the study of photosynthesis, water regime and mineral nutrition, as well as of growth and development of the cotton plant. In a series of reports, problems connected with chromatographic methods and their application in the study of cotton and other plants were presented.

In the field of photosynthesis, there were presented: methods of investigation of the products of this process, problems in measuring radiation energy, a new method of determination of leaf surface, the use of manometric methods, as well as Sachs' method for studying cotton photosynthesis, and other problems.

The reports of the representatives of the Institutes of Genetics and Plant Physiology, AN UzSSR on the problems of water regime and diagnosis of the mineral nutrition of plants were of significant interest. Here one may include the communications concerning methods of determining specific weight and volume of plant tissues, and simple methods of nitrate and phosphate determinations in cotton leaves, and investigations on the irrigation regimes of alfalfa and corn. The report of the representative of NIKhI* about the application of the sap method in agrotechnical experiments with the cotton plant was energetically discussed.

On the problem of development and growth, the representatives of the cotton-growing republics have illuminated in detail the following questions: the significance of ontogenetic and whorl changeability in the physiological study of a plant; the establishment of physiological-biochemical characteristics of the early phase of cotton vegetation; factors accelerating development of the cotton plant; the study of physiological-biochemical characteristics of cotton varieties with different maturation rates; method of measurement of cotton boll bearing; methods of chemical treatment of the cotton plant; methods of study of the physiological-biochemical changes accompanying defoliation.

In the conference, a broad presentation and discussion was devoted to: the use of chromatographic methods and to the application of chromatography in biology; chromatographic methods of determination of gossypiol in various organs of the cotton plant; the quantitative determination of amino acids after their separation by paper chromatography; the quantitative determination of free amino acids and sugars by the method of A. N. Boiarkin. In addition, a report was presented on the quantitative determination of gases in plant sap.

The symposium discussed the plan for scientific work in the physiology and biochemistry of the cotton plant.

*Scientific Research Cotton Institute.

The decision accepted by the conference indicated the necessity for a considerable increase in the theoretical level of physiological-biochemical work on the problem of " nutrition, growth and development of the cotton plant and other crops" and for extending aid of every kind for successful solution of practical problems in the field of cotton production in relation to the decisions of the Congress KPSS. In accordance with this problem, it is planned to investigate in two directions: 1) physiological-biochemical bases for the nutrition, growth and development of the cotton plant and other crops, and 2) the physiological-biochemical basis for the increase of plant resistance to unfavorable conditions.

In the decision accepted, the specific paths and methods of physiological and biochemical investigations of the cotton plant have been outlined in relation to the subdivisions of the problem discussed by the conference, and there were indicated the measures which must be taken for removal of existing deficiencies and for the rapid introduction of modern methods of physiological and biochemical investigation of the cotton plant and other crops in the laboratories of the scientific institutions and universities of the cotton-growing republics.

The participants of the conference have noted the importance and productivity of the first methods conference on the physiology and biochemistry of the cotton plant, which has just taken place. The participation of representatives of the K. A. Timiriazev Institute of Plant Physiology, AN SSSR and of the V. L. Komarov Botanical Institute, AN SSSR was of great significance in the work of the conference.

The decision accepted by the conference expressed the desirability of a subsequent conference on methods in the beginning of 1959.

L. G. Dobrunov

NOTICE

The K. A. Timiriazev Institute of Plant Physiology, AN SSSR, together with the M. V. Lomonosov Department of Soil Biology of Moscow University and the Scientific-Technical Soviet of the Ministry of Higher Education, issue a call for a conference on the physiology of plant resistance (frost resistance, drought resistance and salt resistance) in January 1959.

The purpose of the conference is to sum up the results of investigations on the physiology of drought resistance, winter stability, and salt resistance of plants, and to plan paths for the further development of these problems.

The Organization Committee is inviting institutions to participate in this conference.

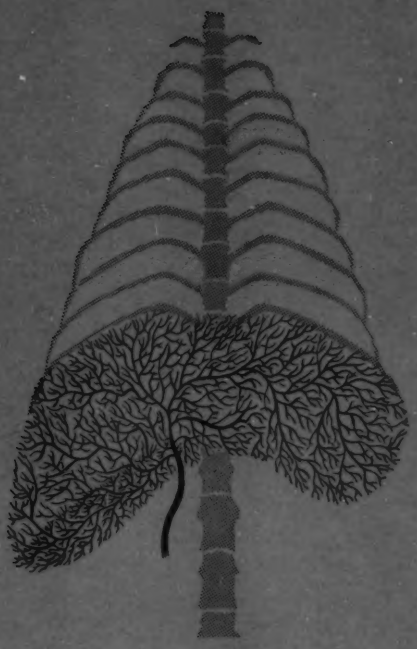
For further information, refer to the Science Secretary of the Organization Committee, Boris Petrovich Stroganov, address:

Moscow I-273, Vladykinskoe shosse, House 15, Artificial Climate Station of the K. A. Timiriazev Institute of Plant Physiology, AN SSSR.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.



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A.I.B.S. Russian Translations

The AIBS is in the process of expanding its Russian Translations Program extensively. Funds to subsidize translation and publication of important Russian literature in biology have been obtained from the National Science Foundation, as part of a larger program to encourage the exchange of scientific information between the two countries. The following monographs have been scheduled for early publication:

Origins of Angiospermous Plants. By A. L. Takhtajian. Edited by G. Ledyard Stebbins. Translated by Olga H. Gankin. 68 pgs. Ready now. $3.00 (U.S. & Canada) $3.50 (Foreign)

Essays on the Evolutionary Morphology of Plants. By A. L. Takhtajian. Edited by G. Ledyard Stebbins. Translated by Olga H. Gankin. Ready November 1958. $5.00 (U.S. & Canada) $5.50 (Foreign)

Problems in the Classification of Antagonists of Actinomycetes. By G. F. Gause. Edited by David Gotdieb. Translated by Fritz Danga. Ready early 1959. $5.00 (U.S. & Canada) $5.50 (Foreign)

X-Rays and Plants. By L. P. Breslavets. Ready early 1959. $5.00 (U.S. & Canada) $5.50 (Foreign)

Arachnida. Vol. IV, No. 2. Fauna of the U.S.S.R. By B. I. Pomerantzev. Edited by George Anastos. Translated by Alena Elbl. Ready early 1959. $10.00 (U.S. & Canada) $11.00 (Foreign)

Arachnoidea. Vol. VI, No. 1. Fauna of the U.S.S.R. By A. A. Zachvatkin. Translated and edited by A. Ratcliffe and A. M. Hughes. Ready spring 1959. $10.00 (U.S. & Canada) $11.00 (Foreign)

Three new Russian journals are being added to the list of four currently translated and published by AIBS:

Soil Science (Pochvovedenie). 12 issues per year. Approx. 1,600 pgs. per year. Will begin with January 1958 issue. Ready January 1959.

Subscriptions: $40.00 per year (General)
$20.00 per year (Libraries of non-profit academic institutions)
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Entomological Revue (Entomologicheskoe Obozrenie). 4 issues per year. Approx. 250 pgs. per year. Will begin with January 1958 issue. Ready January 1959.

Subscriptions: $25.00 per year (General)
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Doklady—Biochemistry Section. 6 issues per year. Approx. 500 pgs. per year. Currently being translated and published by Consultants Bureau, this section of Doklady will now be published by AIBS, beginning with January 1958 issue. Ready September 1958.

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